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Perspective

Current and Novel Approaches to the Drug Treatment of Schizophrenia

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1. Incidence and Clinical Characteristics

Schizophrenia is a devastating psychiatric illness that affects approximately 1% of the world population irrespective of ethnic, economical, or cultural boundaries: features that suggest the disorder has no simple, single causative factor. With current drug therapy, approximately 25% of patients recover to some extent within 5 years of starting treatment and about 65% of patients have recurring problems over many years. The remaining 10−15% of patients develop long-term incapacity and around 15% commit suicide. There are substantial costs, both direct and indirect, incurred by this disorder including those of drug treatment, residential accommodation, physician and other healthcare services, and loss of productivity in the workplace. Clinical symptoms are apparent relatively early in life, generally occurring between the ages of 15 and 45. They are characterized by the presence of positive symptoms, for example auditory hallucinations, disorganized thoughts, delusions, and irrational fears, and negative symptoms, including social withdrawal, diminished affect, poverty of speech, lack of energy, and the inability to experience pleasure. In addition, schizophrenic patients may suffer cognitive deficits including impaired attention, verbal fluency, memory recall, and executive function.

2. Aetiology

The aetiology of the disorder is unknown: subtle structural changes are present in some brain regions although the consistency of these findings between studies is not particularly good (for an extensive, critical review of the neuropathology of schizophrenia see ref 1). Probably the most reliable findings are the small (6%) reduction of brain weight and somewhat variable increase of ventricular volume, although the latter also occurs in other neuropsychiatric disorders and therefore is not considered diagnostic. More recent studies using magnetic resonance imaging (MRI) techniques have confirmed the ventricular enlargement and additionally shown a decrease in the volume of specific brain regions. These include the thalamus[2] and temporal lobe structures including the hippocampal formation,[3] amygdala, and parahippocampal gyrus.[4] Similarly, positron emission tomography (PET) and functional MRI studies have demonstrated that not only are these regions physically affected, they are also implicated in some of the symptoms of the disorder. Thus, specific neuronal circuits or pathways involving the thalamus, caudate-putamen, anterior cingulate, limbic and primary auditory cortex, hippocampus, and parahippocampal gyrus are activated in schizophrenics during auditory hallucinations.[5,6] Cytoarchitectural changes including an alteration in the number, size, or orientation of neurones in several brain regions including the hippocampal formation, thalamus, and entorhinal cortex have been reported, but such findings are inconsistent.[1] At present it is not known how or why these structural changes occur. However, as there is little evidence of glial cell proliferation or associated glial cell proteins, and as the structural changes appear not to be progressive, degenerative mechanisms are not thought to be involved.

Familial studies have shown that the risk of developing schizophrenia is greater in family members than in

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the general population, with the risk being highest in the monozygotic twin of an affected individual. However, a number of monozygotic twin studies also show that the concordance rate for schizophrenia is only approximately 48%, even though twins share 100% of their genes.[7,8] These findings suggest that while there is a strong genetic component to schizophrenia, nongenetic factors, for example environmental, seasonal, socioeconomic, obstetric complications, or viral infection, also play an important role. However, the identification of a single common environmental factor or any genes or loci increasing susceptibility to schizophrenia remains illusive.[9]

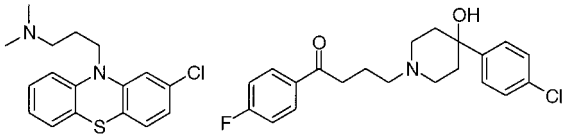
Genetic anticipation (earlier age-at-onset with increased severity of the disease in subsequent generations) is observed in some neurodegenerative disorders, including Huntington's disease and spinocerebellar ataxia, that are associated with expansions encoded by CAG repeats. The severity of such disorders is related to the size of the triplet expansion, which forms expanded polyglutamine containing proteins that aggregate, leading to cell death. The increased risk of schizophrenia in relatives is consistent with genetic anticipation, and it has been suggested that triplet repeat expansions may be involved in the neuropathology of schizophrenia. However, CAG repeat expansions or expanded polyglutamine containing proteins have not been consistently detected in schizophrenics, suggesting either that this mechanism does not contribute to the aetiology of schizophrenia or there is an involvement in only a small population of patients.[10−12] Apoptotic cell death mechanisms involved in nonnecrotic neuronal loss appear not to have been extensively studied in schizophrenic brain tissue. However, preclinical studies have observed apoptotic cell death in brain tissue following blockade of NMDA receptors in foetal and in adult rats.[13−15] These latter findings may be of some relevance if CNS glutamate hypofunction is involved in the aetiology of schizophrenia (see section 3c).

In summary, it is generally thought that schizophrenia is a disorder of developmental maturation rather than neurodegeneration. Structural changes, probably precipitated during pre- or peri-natal life by a convergence of genetic and external factors preferentially, but not exclusively, affect the medial temporal lobe. This brain region is crucially involved in the processing and integration of information from the association cortex, and its dysfunction could be related to some of the observed clinical symptoms which are generally not apparent until early adolescence.

3. Neurochemical Hypotheses of Schizophrenia

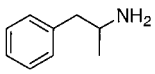
One of the principal difficulties in the development of antipsychotic medication is the fundamental lack of understanding regarding the underlying cause and nature of the disorder. Consequently it is difficult, if not impossible, to design treatment either palliative or restorative on a rational basis. Therefore, in order to facilitate antipsychotic drug development, several neurochemical hypotheses have been developed based on evidence acquired from many sources including biochemical, pharmacological, imaging, electrophysiological, and behavioral studies. While the following is not an exhaustive list of such hypotheses, the examples described serve first to illustrate how they have been used to drive the development of antipsychotic drug treatment and second their relative inadequacy.

a. Dopamine. The discovery that chlorpromazine (**1**) and haloperidol (**2**), known to be clinically efficacious in the treatment of schizophrenia, increased dopamine turnover in rodent brain[16] and possessed high affinity for dopamine D_2 receptors,[17] suggested that schizophrenia is a disorder of increased dopamine function. This was further substantiated by the observation that repeated administration of amphetamine (**3**) in humans



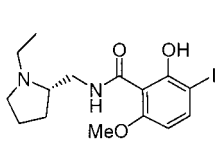
1 Chlorpromazine

2 Haloperidol

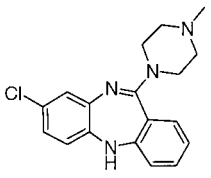


3 Amphetamine

induced paranoid psychosis, with symptoms similar to those observed in some schizophrenic patients. In rodents, amphetamine potently releases both dopamine and noradrenaline in brain and induces catecholamine-dependent behavioral effects (see section 4). These results are consistent with the idea that increased dopamine function contributes toward the psychotomimetic effects of amphetamine. Despite these observations, there has been little direct evidence from neurochemical post-mortem studies to substantiate an abnormality of central dopamine neuronal function in nonmedicated schizophrenic patients. However, recent clinical studies in nonmedicated schizophrenic patients showed that acute administration of amphetamine significantly decreased the striatal binding potential of the dopamine $D_{2/3}$ single photon emission computerized tomography (SPECT) ligand [^{123}I](*S*)-(−)-3-iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide ([^{123}I]IBZM, **4**) to a greater extent than control subjects, suggesting a more pronounced amphetamine induced increase of dopamine release in schizophrenics.[18] Nevertheless, for several reasons including the finding that clozapine (**5**) is an effective antipsychotic drug and yet is a weak dopamine D_2 receptor antagonist at therapeutic doses, it is now clear that the dopamine hypothesis per se, while important as a driving force in the development of antipsychotic treatment, is not sufficient to fully explain such a complex disorder.



4 IBZM



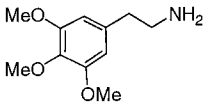
5 Clozapine

Table 1. Atypical Antipsychotics: Human Receptor Binding Profile (Affinity K_i, nM)[a]

	clozapine	risperidone	olanzapine	quetiapine	zotepine[b]	ziprasidone
D_1	53	21	10	390	29	9.5
D_2	190	0.44	2.1	69	11	2.8
D_3[b]	280	14	49	340	6.4	10
D_4[b]	40	16	28	1600	39	39
5-HT_{1A}	710	21	7100	830	330	37
5-HT_{2A}	4.0	0.39	1.9	82	2.7	0.25
5-HT_{2C}	5.0	6.4	2.8	1500		0.55
α_1	3.7	0.69	7.3	4.5		1.9
α_2	51	1.8	140	1100	180	390
M_1	0.98	>5000	2.1	56		>10000
H_1	17	88	5.6	21	0.62	510

[a] Values taken from Arnt et al.[118] [b] Values from Schotte et al.[119]

b. Serotonin. The observation that several serotonin receptor agonists, including mescaline (**6**), bufotenin (**7**), and lysergic acid diethylamide (**8**, LSD), produce hallucinations in humans led to the suggestion that serotonin might be involved in the aetiology of schizophrenia.[19] Furthermore, while early post-mortem studies



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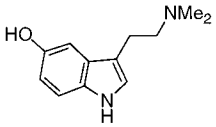
Mescaline



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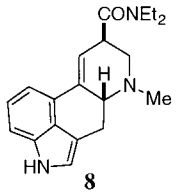
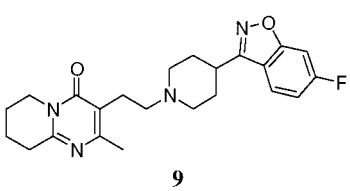
Bufotenin



8

LSD

failed to reveal any major differences in serotonin metabolism, more recent reports have found changes in 5-HT receptors, in particular 5-HT_2 receptors, in the cortex of schizophrenic subjects.[20,21] It was also recognized that the atypical antipsychotic clozapine exhibits high affinity for 5-HT_2 receptors and, in contrast to most antipsychotic drugs, has improved efficacy despite showing low (approximately 40%) dopamine D_2 receptor occupancy at clinically relevant doses. The fact that 5-HT_2 receptors are extensively (85−95%) occupied at comparable doses of clozapine[22] led to the suggestion that preferential blockade of 5-HT_2 versus D_2 receptors may contribute toward its unique clinical profile. However, as chlorpromazine has equally high affinity for 5-HT_2 and D_2 receptors but does not display an atypical antipsychotic profile,[23] it has been suggested that the ratio of 5-HT_2/D_2 rather than the absolute affinity for D_2 and 5-HT_2 receptors may be important for defining an atypical antipsychotic profile.[24] Indeed, a number of the newer atypical antipsychotics including risperidone (**9**) and olanzapine (**10**) demonstrate this particular receptor profile in vitro (see Table 1) and differential 5-HT_2/D_2 receptor occupancy in vivo.[22,25−29] The concept



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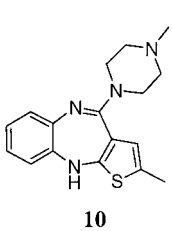
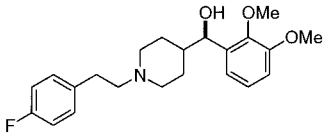
Risperidone



10

Olanzapine

that 5-HT_2 receptor blockade may contribute toward the therapeutic efficacy of clozapine has therefore led to renewed interest in the relevance of 5-HT systems in schizophrenia. The question remains as to whether high affinity for 5-HT_2 receptors per se is sufficient for antipsychotic activity, and this has been recently addressed following the development of the highly selective 5-HT_2 receptor antagonists M 100907 (**11**) and fananserin (**12**) (see section 6b).



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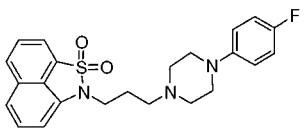
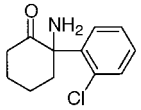
M 100907



12

Fananserin

c. Glutamate. The glutamate hypothesis of schizophrenia suggests that hypofunction of glutamate neurons in the CNS is associated with the pathophysiology of schizophrenia and has been the subject of recent reviews.[30−32] This hypothesis was initially based on the finding that cerebrospinal fluid (CSF) glutamate concentration is reduced in schizophrenia,[33] an observation that has recently been confirmed and extended to show that CSF glutamate concentration correlates inversely with positive symptom severity.[34] Further supportive evidence is provided from post-mortem studies of glutamate receptors, in particular *N*-methyl-D-aspartate (NMDA) receptor density, which, possibly as a consequence of reduced glutamate release,[35] is increased in various brain regions.[36−38] The most compelling evidence is based on the qualitative similarity of schizophrenia-like symptoms in normal volunteers, and the exacerbation of these symptoms in schizophrenics, observed following the administration of the noncompetitive NMDA receptor antagonists ketamine (**13**) or phencyclidine (**14**, PCP).[39,40] While such clinical studies have focused attention on the NMDA receptor, a role for other components of the excitatory amino acid system in schizophrenia remain to be determined.



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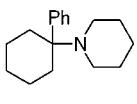
Ketamine



14

PCP

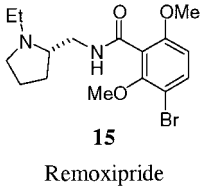
4. Animal Models for Detecting Novel Antipsychotic Drugs

The animal models described in this section are not intended to provide an exhaustive account of those

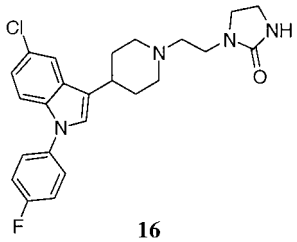
available to identify novel antipsychotic drugs. Rather, selective tests have been chosen which are either commonly used to screen novel compounds or which are of particular interest in that they attempt to model neuropathological and/or neurodevelopmental aspects of the disease and therefore might improve understanding of the disease process.

a. Positive and Negative Symptoms. Given that amphetamine induces effects in humans reminiscent of the positive symptoms seen in schizophrenic patients, it is perhaps not surprising that amphetamine is used preclinically to screen for novel antipsychotic drugs. One of the most obvious behavioral consequences of amphetamine treatment in rodents is a marked stimulation of locomotor activity which is thought to result from an increase in dopamine release in the nucleus accumbens.[41] Given that overactivity in dopaminergic function has been implicated in schizophrenia and that D_2 receptor antagonists are clinically efficacious against the positive symptoms of schizophrenia, blockade of amphetamine hyperactivity is widely used to screen novel drugs. Numerous studies have shown that D_2 receptor antagonists (for example haloperidol), $D_2/5HT_2$ receptor antagonists (for example risperidone), the atypical antipsychotic clozapine, and newer agents such as olanzapine inhibit this response.[42−44]

More recently, attention has shifted toward using the noncompetitive NMDA receptor antagonist PCP, since PCP administration in human volunteers produces effects similar to both the positive and negative symptoms of schizophrenia. In animals, PCP also stimulates locomotor activity, and again this response is blocked by a wide range of established antipsychotics.[45,46] Interestingly, however, PCP treatment has been shown to decrease social interaction in the rat, and it has been argued that this response may represent an animal model of the negative symptoms of PCP psychosis/schizophrenia in humans.[47] Thus, chronic treatment with clozapine, but not haloperidol, significantly reduced this response, consistent with clinical observations that clozapine is uniquely efficacious against negative symptoms in patients.[48] However, recent studies have questioned the predictive validity of the model since a partial reversal of PCP-induced deficits in social interaction were observed with clozapine, remoxipride (**15**), and sertindole (**16**) while haloperidol, risperidone, and olanzapine were ineffective.[49] Some of these latter agents are reported to improve negative symptoms in humans although it has been argued that clinical trials may not adequately distinguish improvements in primary negative symptoms from improvements reflecting the reduced extrapyramidal symptom (EPS) liability of newer agents. Chronic PCP administration has also been reported to increase immobility in the mouse Porsolt swim test,[50,51] a well-established behavioral response which, in non-PCP treated mice, is reduced by antidepressant drugs. While antipsychotics have negligible effects on immobility per se, pretreatment with risperidone, clozapine, or the $5\text{-}HT_{2A/2C}$ receptor antagonists mianserin (**17**) and ritanserin (**18**) attenuated the increase in immobility induced by PCP. In contrast, haloperidol had no effect, suggesting that PCP enhanced immobility may be a model of negative symptoms and that $5\text{-}HT_2$ receptor antagonists may be efficacious in treating negative symptoms in schizophrenic patients.



15
Remoxipride



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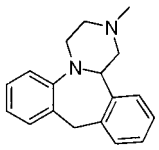
Sertindole



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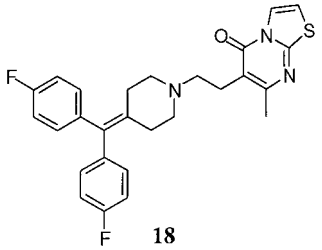
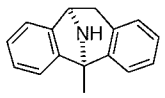
Mianserin



18
Ritanserin

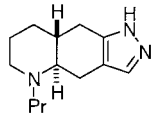
Consistent with the well-characterized behavioral effects of PCP, mutant mice with reduced NMDA receptor expression (5−10% of normal) have also been reported to show increased locomotor activity, increased stereotyped behavior, and a decrease in social interaction.[52] Furthermore, while administration of either PCP or MK-801 (**19**, a more selective noncompetitive NMDA receptor antagonist) did not enhance locomotor or stereotyped responses in mutant mice, wild-type mice showed a marked increase in both behaviors to a level similar to that seen in untreated mutant animals. Consistent with the effects seen in pharmacological studies, treatment with haloperidol or clozapine dose dependently and significantly attenuated the hyperactivity seen in NMDA receptor knock down mice, and in the case of clozapine, this occurred at doses some 40 times lower than those reducing spontaneous locomotor activity in wild-type mice. NMDA receptor mutant mice also showed reduced social investigation in a resident intruder paradigm which was attenuated following clozapine treatment. Clearly these animals are of interest, and further examination of their behavioral phenotype and the effects of established antipsychotics will establish their value over and above the pharmacological approaches commonly used for identifying novel treatments.



19
MK 801

In addition to the locomotor enhancing effects of PCP and amphetamine, considerable attention has focused on the ability of these agents to disrupt prepulse inhibition. Essentially, prepulse inhibition is a model of sensorimotor gating which can be assessed in both animals and humans using the startle reflex response; when a fixed startle eliciting stimulus (i.e., the pulse) is preceded by 30−500 ms by a weak, nonstartle eliciting

stimulus (i.e., the prepulse), the magnitude of the startle response is significantly reduced compared to that recorded to the pulse alone.[53] Prepulse inhibition occurs whether the prepulse or pulse are of the same or different modalities (e.g., tones and/or air puffs), is present on the first exposure (i.e., is not a form of conditioning) and does not habituate over successive trials, and is thought to represent preattentive filtering mechanisms. Deficits in prepulse inhibition are also apparent in schizophrenic patients, which may relate to their inability to adequately inhibit (or "gate") irrelevant sensory information which results in sensory "flooding" and cognitive fragmentation. In animals, prepulse inhibition is disrupted by both amphetamine[54] and by directly acting D_2 receptor agonists (for example quinpirole (**20**)[55] and apomorphine (**21**)[56]) and by PCP;[57] however, while D_2 receptor antagonists can reverse the deficits induced by amphetamine and apomorphine,[58] these compounds do not reverse the effects of PCP.[59] In contrast, the atypical neuroleptic clozapine,[60] and the newer agents olanzapine[61] and quetiapine (**22**),[62] are effective at reducing PCP-induced deficits in prepulse inhibition, suggesting that this paradigm is a useful screen for "atypical" antipsychotics. Prepulse inhibition is also impaired in rats reared in social isolation following weaning,[63] an effect which may result from enhanced dopaminergic activity.[64] Again, this deficit is attenuated by treatment with quetiapine, olanzapine, haloperidol, clozapine, and risperidone, suggesting the model is able to detect a wide range of different antipsychotic drugs.[65,66]



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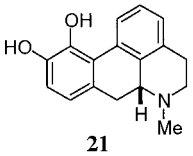
Quinpirole



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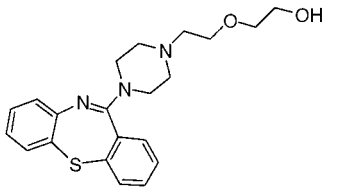
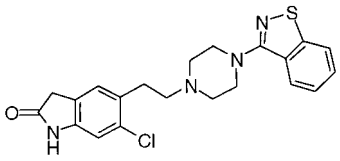
Apomorphine



22
Quietapine

A third model commonly used to predict antipsychotic efficacy is inhibition of conditioned avoidance responding. There are several variations of this test, but generally testing is carried out in shuttle boxes which are divided into two equal compartments connected via an open doorway. Each compartment is equipped with a light source which signals the impending delivery of a mild footshock; animals are trained to avoid shocks by crossing into the opposite chamber during light presentation (i.e., the conditioning stimulus) which prevents shock delivery. Thereafter crossing after initiation of the shock terminates shock delivery and is recorded as an escape response. In animals trained to perform this task, treatment with antipsychotic drugs reduces conditioned avoidance responses without impairing the animals ability to escape, whereas other psychoactive agents, for example antidepressants, reduce both avoidance and escape responses.[67] A wide range of antipsychotic drugs are effective in this test including D_2 receptor antagonists,[68] clozapine,[69] risperidone,[70] and newer drugs such as olanzapine[71]and ziprasidone (**23**).[72]



23
Ziprasidone

b. Side Effect Liabilities. As described below, one of the major problems associated with chronic treatment with D_2 receptor antagonists is the onset of EPS. These are thought to result from blockade of striatal D_2 receptors which, in rodents, produces a cataleptic behavioral response. This is typically assessed by placing the animal in an abnormal posture such as placing the animal's forepaws over a circular metal bar raised above the bench surface and recording how long the animal will hold this abnormal position. Vehicle-treated animals will immediately normalize their posture while animals treated with D_2 receptor antagonists will maintain the position for several minutes. In contrast, the atypical antipsychotic, clozapine, fails to produce catalepsy,[73] and some of the newer agents, for example olanzapine, appear to exhibit a window between doses effective in assays predictive of antipsychotic potential and those required to induce a measurable cataleptic response.[71] A second approach commonly used to determine the extent of blockade of striatal dopaminergic function is to establish whether novel agents will block the induction of stereotyped behavior induced by dopamine receptor agonists such as apomorphine. This response consists of repetitive sniffing, licking, and biting and is known to result from activation of striatal dopamine receptors.[74−76] Again D_2 receptor antagonists will inhibit this response whereas the atypical antipsychotic, clozapine, has negligible effects,[77] consistent with the clinical observation that clozapine has reduced EPS liability.

A second side effect liability which can easily be detected in preclinical studies is the likelihood of inducing sedation. In animals this is usually assessed in tests of motor activity such as placing animals in a novel environment, for example photocell activity cages.[77] Initially exploratory activity is high but animals pretreated with sedatives will show less exploratory behavior when they are first placed in cage. It is important to ensure that an adequate pretreatment time has been allowed so that good plasma/brain drug levels are achieved prior to testing the animal, since maximum effects on motor activity are only detected in approximately the first 10 min, after which activity declines as animals habituate to their environment. A second approach is to examine effects on a rotarod apparatus which may be revolving at a fixed speed, for example 15 revolutions per minute, or may accelerate as the test progresses.[77] Again, drugs producing seda-

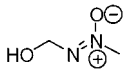
tion or impairment of motor coordination will significantly reduce performance in this test. Finally, it is well established that D_2 receptor antagonists will stimulate prolactin secretion both in humans and rodents,[78] and the effects of novel agents are usually examined in rodents prior to clinical assessment.

c. Neurodevelopmental/Degeneration Models. While many studies examining the antipsychotic potential of novel drugs rely on the use of PCP and amphetamine, it is clear that this approach makes no attempt to model either the neuropathology or developmental aspects of the disease. There are, however, models described in the literature which attempt to address some of these issues although such assays do not appear to be routinely used within the pharmaceutical industry. One such model is the neonatal excitotoxic hippocampal lesion.[79] Essentially, 7 day old (postnatal day 7 (PD7)) rat pups are anesthetized and stereotaxically injected with either vehicle or ibotenic acid into the ventral hippocampal formation and then returned to their mothers until weaning (PD25). When the brains from mature animals are examined, this procedure is shown to produce neuronal loss, atrophy, neuroglial proliferation, and some cavitation in the ventral hippocampus (CA1, CA3, dentate gyrus, subiculum) while sparing the dorsal hippocampus. Cell loss appears to be predominantly restricted to the ventral hippocampus although slight damage to adjacent thalamic areas, amygdaloid nuclei, and to the perirhinal and entorhinal cortex has occasionally been observed.[80] Behavioral testing is carried out either prepuberty (PD35) or postpuberty (PD56), and at PD56 lesioned animals are reported to show increased spontaneous exploration and increased amphetamine-induced hyperactivity. What is more, lesioned rats show a significant increase in locomotor activity following swim stress, suggesting that these animals are hyper-responsive to stressors. Subsequent studies have also demonstrated that neonatally hippocampal lesioned rats show deficits in prepulse inhibition,[81] reduced social interaction,[82] and impaired performance on the radial arm maze[83] (a hippocampal-dependent spacial working memory task) although only the deficit in prepulse inhibition appears to emerge postpuberty. In vivo microdialysis studies[84] clearly show that the exaggerated locomotor response to amphetamine does not result from an increase in amphetamine-induced dopamine release in the nucleus accumbens. Together with the fact that direct acting dopamine receptor agonists (for example quinpirole) also enhance locomotor responses in lesioned rats,[80] this suggests that postsynaptic D_2-like receptor supersensitivity occurs. Interestingly, potassium stimulated [^{3}H]-D-aspartate release was significantly reduced in hippocampal and frontal cortical slices from mature rats receiving neonatal hippocampal lesions, suggesting that, in addition to a hyperactive dopamine system, these animals exhibit glutamate hypofunction.[85] Indeed, a significant increase in specific [^{3}H]glutamate binding was reported in frontal cortex, suggesting a compensatory increase in glutamate receptor number. Clearly the behavioral effects observed following neonatal hippocampal lesions, the fact that a number of these emerge post puberty, and the restriction of cell loss to the ventral hippocampus (although it should be noted that this is substantial compared to the subtle abnormalities observed in schizophrenic patients) make this model attractive. The key question is whether any of these behavioral effects can be attenuated following antipsychotic drug administration. Limited studies to date have shown that chronic treatment with either clozapine or haloperidol attenuates the spontaneous hyperlocomotion seen when lesioned rats are placed in a novel environment, although both compounds are sedative per se and, at least in the case of haloperidol, reduced motor responses were also recorded in the sham lesioned rats.[86] Also, the deficits in social interaction observed in lesioned rats, a response thought to model components of the negative symptoms of schizophrenia, were actually worsened in clozapine-treated lesioned animals,[82] in contrast to the beneficial effects on negative symptoms seen in the clinic. In our own studies[87] we have focused on the deficits in prepulse inhibition which emerge postpuberty and are not seen when adult rats are lesioned in the ventral hippocampus. We have systematically examined a wide range of typical, atypical, and putative antipsychotic agents and have failed to observe any effect on prepulse inhibition following acute drug administration. Clearly the effects of chronic treatment should be considered, but to date the ability of any antipsychotic drug to convincingly attenuate behaviors in neonatally lesioned rats has yet to be demonstrated.

An interesting alternative approach, which also attempts to model the neuropathology seen in the brains of schizophrenic patients, is a kainic acid lesion performed in adult rats.[88] When administered into the cerebroventricles, CA3 and CA4 hippocampal neurons appear to be selectively vulnerable to the neurotoxic effects of kainic acid, most likely because large numbers of kainate receptors are found in this region. Neuronal regrowth also occurs, and redundant cell connections may develop in the dentate and CA1 hippocampal areas within days of kainic acid infusion. Thus this approach may be relevant to schizophrenia in that both show moderate levels of cell loss in CA3/CA4 areas, aberrant reinnervation and/or axonal disarray is a feature of the pathology of both, and a reduction in hippocampal kainate receptors has been reported in post-mortem studies of schizophrenic brains.[89] In rats, increased behavioral sensitivity to stress and dopamine receptor agonists has been reported[90] which may relate to an increase in dopamine D_2 receptors reported in the nucleus accumbens.[91] Again limited studies have attempted to examine the effects of established antipsychotic drugs, although the ability of haloperidol, but not clozapine, to block dopamine receptor agonist-induced hyperlocomotion is impaired in kainic acid lesioned rats,[92] suggesting that the model may mirror aspects of the treatment-resistant effects seen in some schizophrenic patients treated with classical neuroleptics.

A model which attempts to mimic some of the neurodevelopmental aspects of schizophrenia involves treating pregnant rats with the alkylating agent methylazoxymethanol (**24**, MAM) which, in embryos, kills mitotically active cells through methylation of nucleic acids.[93] When administered at embryonic day 15 (E15), adult animals show marked microencephaly with a 40−50% reduction in weight of the cortex, striatum, and

hippocampus,[94] and these animals show deficits in



24

Methylazoxymethanol

learning and memory tests, increased sensitivity to convulsive agents,[95] and increased locomotor activity.[96] Clearly, such gross pathology is not observed in schizophrenia, and more recent studies have attempted to produce more subtle anatomical changes following MAM treatment.[97] These have focused on producing localized damage within the entorhinal cortex which is involved in processing multimodal, associative information and channeling it to the hippocampus and which shows subtle anatomical changes (reductions in cell number, volume, and/or neuronal disorganization) in schizophrenia. Given that MAM is short acting and is only effective in cells actively proliferating, it has been administered at E9 to E12, when the entorhinal cortex is developing but which should avoid the gross microenchephaly produced at E15. Consistent with these proposals, adult brain weights were normal in animals treated at E9 to E11 or were only slightly (6%), but significantly, reduced in animals treated at E12. At E9 to E11 the morphological consequences in the adult rat brain were largely restricted to the entorhinal cortex, although slight effects were observed in the frontal and occipital cortex and these included cortical thinning and disorganized cortical layering. More widespread changes were noted in animals treated at E12, including abnormalities in the caudal hippocampus and amygdalohippocampal area. To date only limited information is available concerning the behavioral phenotype of these rats, although adult animals exposed to MAM at E9 to 12 appear normal in tests of social interaction, open field, and novel object exploration.[98] In contrast, E11 and E12 MAM treated rats show retardation in passive avoidance acquisition although the latter also exhibit reduced pain sensitivity which may influence detection of mild electric footshocks.[98] Whether these animals show deficits in prepulse inhibition or are hyper-responsive to stressors or psychostimulants remains to be addressed and the utility of this approach for examining antipsychotic drugs unknown.

d. Transgenic Mice. A number of transgenic mice have now been generated which show anatomical and/or behavioral characteristics, for example impaired prepulse inhibition, reminiscent of those seen in schizophrenic patients. While it should be stressed that it is currently unclear whether established antipsychotic drugs can modify any of these behavioral effects, the hope is that these animals will assist in further understanding the aetiology of the disease and in the identification of better therapeutic agents. One of the first such animals to be described was the *Dvl*1 mutant mouse[99] which is deficient in one of the three mouse homologues of the Drosophila segment polarity gene, *Dishevelled*. While the function of these genes is unknown, in Drosophila, *Dishevelled* is involved in the wingless/Wnt pathway which is a highly conserved developmental pathway involved in cell fate determination in virtually all eukaryotic organisms. *Dvl*1 deficient mice are viable, fertile, and do not show any major structural abnormalities of the brain. However, they do exhibit a distinct behavioral phenotype: mutant mice show marked decreases in social interaction as evidenced by decreased nest building, reduced social contact during sleep, reduced whisker trimming of littermates, subordinate responses in a social dominance test, and a deficit in prepulse inhibition of the acoustic startle response. In contrast, motor performance, pain sensitivity, and cognitive function (assessed using the Morris water maze) are similar to wild-type mice. Given that sensorimotor deficits and social isolation are often seen in schizophrenic patients (and also in schizotypal personality disorder, Tourettes syndrome, and obsessive compulsive disorder) these animals may provide a useful animal model to study factors/therapies influencing these disorders.

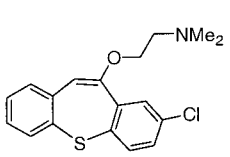
Two additional mutant mice may also be of interest because their behavioral phenotype is associated with morphological changes in discrete brain regions. NCAM-180 knockout mice are reported to show increased lateral ventrical size and deficits in prepulse inhibition, differences similar to those seen in schizophrenic patients.[100] NCAM (neural cell adhesion molecule) is a cell surface receptor belonging to the immunoglobulin superfamily and plays an important role in neuronal migration, neuronal connectivity, and the stabilization of cell contacts and synapses. While encoded by a single gene, the protein is expressed in a number of polypeptide variants following alternative splicing and also undergoes posttranslational modifications leading to the addition of several α-2,8 sialic acid residues on its extracellular domains. Polysialic acid rich NCAM (PSA-NCAM) can enhance the neurite growth promoting activity of NCAM, and a marked reduction in PSA-NCAM levels in hippocampus has been reported in schizophrenic brain. While the findings in NCAM-180 mutant mice are clearly of interest, it should be noted that the main anatomical differences are seen in the olfactory bulbs,[101] where decreased bulb size is associated with a marked reduction in the number of granule cells and disturbed cell organization. In addition, in adult mutant mice, reductions in the NCAM-140 isoform were also apparent, indicating that the mutation may not be specific for NCAM-180. The second mouse of interest is the heterozygous *reeler* mouse which carries a single mutant allele for the *reeler* gene which normally encodes a protein, reelin.[102] Reelin is crucially involved in embryonic corticogenesis by guiding migration and positioning of neurons. Homozygous mice, which completely lack the expression of reelin, show gross abnormalities in neuronal organization in the cortex, hippocampus, cerebellum, and olfactory bulbs.[103,104] These animals also show impaired motor coordination, tremor, and ataxia which emerge on postnatal day 14 and persist into adulthood. In contrast, heterozygous rl -/+ mice show about a 50% decrease in brain levels of reelin mRNA and protein and are indistinguishable from wild-type mice in terms of motor behavior. More importantly, these mice appear to show more subtle defects in neuronal organization in the medial prefrontal cortex and cingulate gyrus whereas other brain regions, including cerebellum, are apparently normal. These ani-

mals also exhibit age-dependent deficits in prepulse inhibition (significant at ages of 55 days and older) and augmented anxiety-like behaviors on the elevated plus maze. They are clearly of great interest given that reduced levels of reelin have been reported in postmortem schizophrenic brain (prefrontal cortex and temporal cortex, hippocampus, caudate nucleus, and cerebellum). Future studies confirming the subtle anatomical defects and exploring the effects of established antipsychotics will clearly be important in determining whether these mice will ultimately be useful for identifying novel antipsychotic drugs.

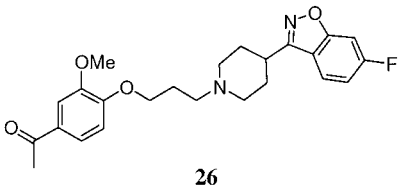
5. Current Therapy

While it is apparent that dopamine D_2 receptor antagonists are effective in the management of psychosis, such compounds, typified by chlorpromazine and haloperidol, are mainly effective in the treatment of positive symptoms and, significantly, have little impact on negative symptoms or cognitive deficits. Furthermore, chronic treatment of patients with such compounds may result in the onset of Parkinson's disease-like EPS, acute dystonia, and with repeated dosing, an increasing risk of tardive dyskinesia that is potentially irreversible. In addition, blockade of dopamine D_2 receptors leads to an increase in prolactin secretion and associated neuroendocrine disorders including gynecomastia, amenorrhoea, and sexual dysfunction.[105] Not surprisingly, these mechanism-based side effects have led to poor patient compliance and the need to find improved treatments.

The experience with clozapine shows that it is possible to make a drug that has a lower propensity to cause EPS than classical neuroleptics,[106] treat patients who are unresponsive to other drugs,[107−109] and possibly have a beneficial effect on the cognitive deficits associated with schizophrenia,[110] an often ignored aspect of the disease. Generally, results on the efficacy of antipsychotics on negative and cognitive symptoms are confounded by both antipsychotic induced EPS and superimposed depressive syndromes. Claims for the newer agents in this regard should therefore be viewed cautiously. Nevertheless, clozapine is effective in approximately 30% of patients who are not successfully treated with typical antipsychotics, i.e., treatment resistant or refractory patients. Furthermore, the atypical antipsychotics have a low propensity to induce EPS and elevate prolactin secretion, making them more acceptable for chronic administration. The decreased level of EPS associated with clozapine and some of the newest agents may account for why some patients appear happier and more social while receiving these agents. Unfortunately, clozapine's clinical utility is limited by its toxicity, producing agranulocytosis in a small proportion of patients. As a consequence, haematological monitoring of patients is essential and adds to treatment costs. After clozapine there have appeared a number of new atypical antipsychotics, including risperidone,[111] olanzapine,[112] quetiapine,[113] ziprasidone,[114] zotepine[115] (**25**), iloperidone[116] (**26**), and sertindole.[143] All are clinically efficacious with regard to positive symptoms and most reduce the incidence of negative symptoms although whether they will also be effective in refractory patients remains to be seen.



25
Zotepine

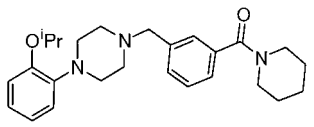


26
Iloperidone

6. Atypical Antipsychotics: Recent Approaches

The newer antipsychotics are clearly effective in treating schizophrenic symptoms and have the added advantage over clozapine that they do not induce agranulocytosis. However, they do exhibit a variety of other side effects,[120−122] which are probably related to their significant affinity for numerous other receptors (Table 1). These include weight gain (5-HT_{2C} receptor blockade), postural hypotension, sedation and dizziness (α_1 adrenoceptor blockade), dry mouth (muscarinic M_1 receptor blockade), and sedation (histamine H_1 receptor blockade). In addition, activity at the IKr channel, a voltage gated potassium channel in the heart[123] which is involved in the control of heart rhythm, is a liability that has resulted in the recent withdrawal of sertindole because of increased risk of prolonging the QT interval and thereby producing cardiac arrhythmias.[124,125]

Despite the introduction of new drugs during the past decade, and the reintroduction into clinical usage of clozapine, the need clearly remains for treatments that are more efficacious and have fewer side effects than those currently available. Unfortunately clozapine has an extremely broad range of pharmacological activities, and deciding which of these is relevant to its beneficial effects is not, a priori, possible. One partially successful approach to mimicking the properties of clozapine has been to combine dopamine D_2 receptor affinity with affinity at another receptor. Many of the newer antipsychotics described above were designed using the rationale described in reference 24 which proposes that it is the ratio of 5-HT_2/D_2 receptor affinities that confers atypicality. Another approach has been combination of D_2 receptor antagonism with $5HT_{1A}$ receptor agonism,[126,127] and a compound with this profile, mazapertine (**27**),[128] has been into clinical trials in humans,

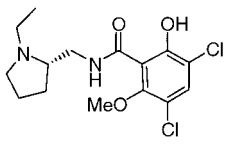


27
Mazapertine

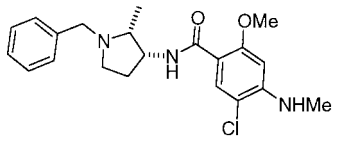
where it was shown[129] to cause postural hypotension, probably as a result of its affinity at α_1 adrenoceptors. These two cases highlight an inherent problem with targeting more than one receptor in a drug discovery project. Because those two receptors have different structure−activity relationships for binding (and efficacy), it becomes an extremely difficult problem to exclusively target those receptors without building in activity at other receptors, particularly when pharmacokinetic and brain penetration parameters also need to be optimized. The outcome can be seen in the side effect profiles of these drugs.

Notwithstanding the promiscuous binding profile of clozapine, a second approach, which has been the focus of much of the drug discovery effort on schizophrenia for the past decade, has been to target a single receptor at which clozapine has high affinity. While conceptually and practically more simple, this approach assumes that activity at one receptor is sufficient to convey the clinical properties of this agent. Two examples of this approach over recent years have been the development of selective dopamine D_4 receptor antagonists and selective 5-HT_{2A} receptor antagonists.

a. Dopamine D_4 Receptor Antagonists. While the antipsychotic efficacy of typical neuroleptics has been ascribed to dopamine D_2 receptor blockade, it is now clear that the D_2 receptor is one of a family of G protein coupled receptors that also includes the D_3[130] and D_4[131] receptor subtypes. Not only do these subtypes show high sequence homology and coupling to inhibition of adenylyl cyclase activity, they also exhibit similar pharmacologies, and many existing antipsychotics have high/modest affinity for all three receptor subtypes. This has led to speculation regarding the contribution of each subtype toward the side effect liability, to the clinical efficacy of antipsychotic drugs, and to the development of subtype selective agents. In particular, considerable effort has been spent developing D_4 receptor antagonists following the observation that clozapine has an order of magnitude higher affinity for cloned human (h) D_4 receptors than hD_2 receptors and therefore the suggestion that D_4 receptor antagonism might contribute toward its unique clinical profile. Interest was further fueled by studies comparing D_2 and D_4 receptor affinity with plasma concentrations of antipsychotic drugs showing that, at clinically relevant doses, clozapine could preferentially occupy dopamine D_4 receptors in the brain[132] (but note that the same argument could also implicate 5-HT_{2A}, 5-HT_6, 5-HT_7, α_1 adrenoceptors, histamine H_1 receptors, and muscarinic receptors). Additional evidence also suggested that D_4-like receptors were increased in the striatum of schizophrenic brain,[133−136] although these results have proved somewhat controversial and have not been replicated by other groups.[137,138] Furthermore, the indirect methods used to identify D_4-like receptors, based on the subtraction of [^{3}H]-raclopride (**28**) binding (to D_2 and D_3 receptors) from [^{3}H]-nemonapride (**29**) binding (to D_2, D_3 and D_4 receptors) are problematic, in that nemonapride binds to additional sites (5-HT_{1A}, σ, and 5-HT_{2A} receptors)[139−141] which may not have been adequately masked and because selective D_4 antagonists were not available to confirm that this residual binding was indeed to the D_4 receptor. More recently, D_4 receptors have been visualized in human brain using the selective D_4 radiolabel [^{3}H]-NGD-94-1 (**30**).[142] These studies have confirmed that the D_4 receptor is present in low abundance, with highest levels detected in the hippocampus and entorhinal cortex and very low levels detected in the striatum and nucleus accumbens. In contrast to earlier work, [^{3}H]-NGD-94-1 binding was not elevated in the striatum from schizophrenic patients. Interestingly, D_4 receptor number in the CA1 area of the hippocampus and entorhinal cortex was increased, and this did not appear to be related to antipsychotic medication. Thus, while present in low density, D_4 receptors are located in cortical and limbic areas classically associated with schizophrenia, and blockade of these receptors could contribute toward the clinical efficacy of clozapine. In addition, selective D_4 receptor antagonists would not be expected to produce EPS and hyperprolactinaemia which are thought to result from dopamine D_2 receptor blockade.



28
Raclopride



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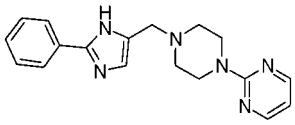
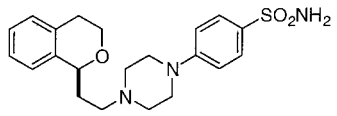
Nemonapride



30
NGD-94-1

On the basis of this evidence, many pharmaceutical companies launched major efforts aimed at discovering high affinity, selective dopamine D_4 receptor antagonists. Among the compounds which have been disclosed to date is NGD-94-1 (**30**),[143,144] a high affinity (K_i 3.6 nM), selective (>600-fold over D_2) D_4 receptor antagonist synthesized by Neurogen which progressed into phase 1 clinical trials in humans. In addition, UpJohn has identified the sulfonamide U-101,387 (sonepiprazole, **31**; D_4 K_i 6.8 nM and 600-fold selective over D_2 receptors),[145,146] and Parke-Davis developed PD-172938 (**32**; D_4 K_i 7.8 nM)[147] and more recently the chromenone (**33**; D_4 K_i 5.8 nM and 500-fold selective over D_2 receptors).[148] High affinity D_4 receptor antagonists have also been disclosed by Servier (S 18126, **34**; D_4 K_i 2.4 nM and >300-fold selective over D_2)[149] and Pfizer (CP-293019, **35**;[175] D_4 K_i 3.4 nM and >970-fold selective over D_2).[150]



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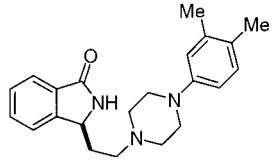
U-101,387



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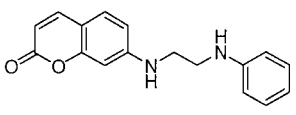
PD-172938



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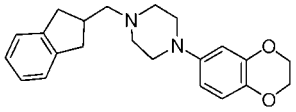
PD-chromenone



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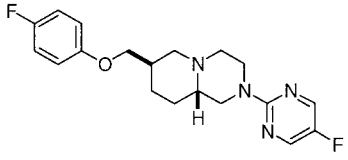
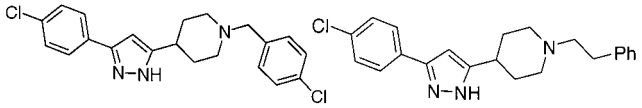
S-18126

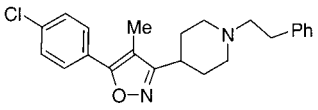


35
CP-293019

We focused on two lead series of compounds, both derived from leads obtained by screening of the sample collection. Using the topological similarity probe method[151] as implemented in our in house TOPOSIM program,[152] the Merck sample collection was screened for compounds with affinity for dopamine receptors and binding selectivity for D_4 over D_2 receptors. One of these searches, based on the structure of haloperidol, provided the pyrazole (**36**)[153] with moderate affinity (K_i 61 nM) and binding selectivity (4-fold) for D_4 receptors over D_2. It was shown that homologation of the benzyl group to phenethyl (**37**) gave a compound with higher affinity (K_i 5.5 nM) and now 100-fold D_4/D_2 selectivity. Changes to the pyrazole led to the discovery of the isoxazole (**38**), a

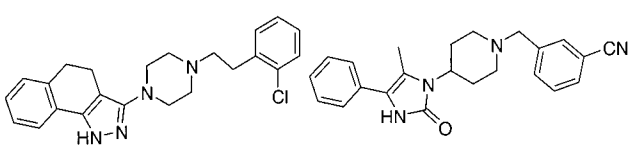


36 **37**



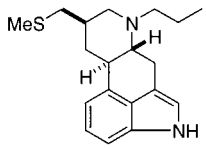
38
L-741742

D_4 receptor antagonist (K_i 3.5 nM) with even higher selectivity over D_2 (>500-fold) and D_3 (200-fold) receptors and good rat pharmacokinetics (F 38%, $t_{1/2}$ 2 h). Mindful, however, of the problems with binding to ion channels often seen in neuroleptics, it was found[154] that **38** had high affinity for sodium, calcium, and potassium channels. This binding was found to be related to the pK_a of the basic nitrogen, with more basic compounds having higher affinities. Introduction of a further nitrogen atom into the aliphatic heterocycle to reduce the pK_a, along with a conformational restriction to increase affinity, led to the piperazinylbenzindazole (**39**) with a similar dopamine receptor binding profile to **38** (D_4 K_i 4.3 nM, selectivity over D_2 >400, D_3 >1000) but low affinities at calcium and sodium channels (58 and 33% inhibition of binding at 10 μM, respectively). Again this compound had acceptable rat pharmacokinetics (F 31% $t_{1/2}$ 1 h) although rhesus pharmacokinetics was poor (F 3%, $t_{1/2}$ 1 h). An alternative[185] strategy to lowering the pK_a was to make the aromatic heterocycle more electron withdrawing. This led to the imidazolidone (**40**) with subnanomolar hD_4 affinity, excellent selectivity over other dopamine receptors (>2000-fold over hD_2, >4000-fold over hD_3), and no significant ion channel activities. Compound **40** had good rat pharmacokinetics (F 69%, $t_{1/2}$ 1.1 h) and occupied central receptors after oral dosing (for a discussion of this issue, see below).

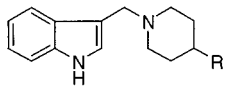


39 **40**

The second TOPOSIM search of the Merck collection was based on the nonselective dopamine receptor agonist Pergolide (**41**), giving rise to the lead structure (**42**) with modest (K_i 570 nM) D_4 affinity and 10-fold selectivity over D_2.[156] The compound (**43**) without the methyl group on the piperidine did not bind to D_4 receptors, suggesting that increased lipophilicity at this point may be beneficial. This, indeed, turned out to be the case, with the phenylpiperidine (**44**) having 10-fold improved

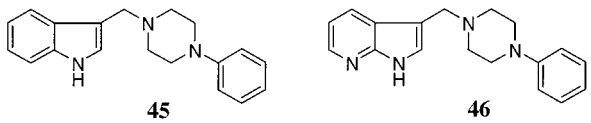


41
pergolide

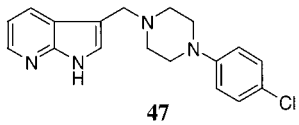


42 R = Me
43 R = H
44 R = Ph

affinity (D_4 K_i 25 nM), although this compound had reduced selectivity over D_2 (4-fold) and high affinity at sodium channels[154] (100% displacement of [^{3}H]-batrachotoxin binding to rat cortical membranes at 1 μM). As for the other Merck series, introduction of an extra nitrogen atom, to give piperazine (**45**) helped reduce this problem, leading to a compound with increased D_4 affinity (K_i 8 nM), selectivity (15-fold over D_2), and an IC_{50} > 10 μM at Na channels. A further improvement of in vitro properties was found with the introduction of a nitrogen atom at the 7-position of the indole (**46**) (D_4 K_i 1.9 nM, D_2/D_4 selectivity 160-fold), although this compound had poor rat pharmacokinetics (C_{max} 18 ng/mL after 3 mg/kg p.o.). Introduction of 4-substituents onto the benzene ring of the phenylpiperazine improved the pharmacokinetics greatly, and the compound taken for further development, L-745870 (**47**), had good bioavailability in rats (F 66%) and rhesus (F 20%) and a half-life of 2−3 h in both species.



45 **46**



47
L-745870

L-745870 is a high affinity (K_i 0.43 nM), selective (D_2/D_4 2000-fold, D_3/D_4 5000-fold) ligand at hD_4 receptors[157] which exhibited antagonist activity in several in vitro functional assays. These included (i) inhibition of dopamine-mediated inhibition of forskolin stimulated cAMP production in HEK and CHO cells expressing hD_4 receptors; (ii) blockade of dopamine mediated stimulation of [^{35}S]GTPγS binding; (iii) inhibition of dopamine mediated increases in acidification rates in CHO cells expressing hD_4 receptors; (iv) blockade of dopamine-induced inhibition of Ca^{2+} currents in GH4C1 pituitary cells; and (v) blockade of G-protein gated inwardly rectifying K^+ currents in *Xenopus* oocytes.[158] In none of these assays was there any evidence of intrinsic efficacy, although an isolated report has suggested that L-745870 may have partial agonist activity.[159] The significance of this result is unclear since partial agonist activity has only been observed in artificial cell lines expressing high D_4 receptor densities (>560 fmol/mg

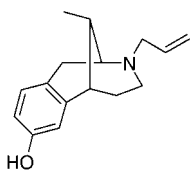
Table 2. Preclinical Profile of Selective D_4 Receptor Antagonists in Behavioral Tests Predicting Antipsychotic Potential and Side Effect Liabilities[a]

		L-745870	U-101,387	S 18126	CP 293019
antipsychotic potential	Blockade of apomorphine or amphetamine hyperactivity	ED_{50} = 22 (mg/kg, p.o.)	NE @ 58 (μmol/kg, i.p.)	ED_{50} = 9 (mg/kg, s.c.)	ED_{50} = 13 (mg/kg, p.o.)
	Blockade of apomorphine disruption in PPI	NE@ 1 (mg/kg, p.o.)	MED = 30 (mg/kg, s.c.)		MED = 18 (mg/kg, s.c.)
	Inhibition of conditioned avoidance responding	NE @ 1 (mg/kg, i.p.)		NE @ 40 (mg/kg, s.c.)	
EPS liability	Blockade of stereotypy	NE @ 1 (mg/kg, p.o.)			NE @ 56 (mg/kg, p.o.)
	Induction of catalepsy	MED = 100 (mg/kg, p.o.)	NE @ 58 (μmol/kg, i.p.)	NE @ 80 (mg/kg,.s.c.)	NE @ 56 (mg/kg, p.o.)
motor impairment	Impaired rotarod performance	MED = 100 (mg/kg, p.o.)	NE @ 60 (μmol/kg, i.p.)	ED_{50} = 66 (mg/kg, s.c.)	
	Impaired spontaneous locomotor activity	MED = 30 (mg/kg, p.o.)	NE @ 58 (μmol/kg, i.p.)	MED = 40 (mg/kg, s.c.)	NE @ 56 (mg/kg, p.o.)
increased plasma prolactin		NE @ 10 (mg/kg, p.o.)	NE @ 25 (μmol/kg, s.c.)		

[a] Abbreviations: ED_{50} = dose inhibiting the response by 50%; MED = minimum effective dose; NE = not effective; EPS = extrapyramidal side effects; s.c. = subcutaneous administration; p.o. = oral administration; i.p. = intraperitoneal administration.

protein)[160] which are very much in excess of those reported in human brain or schizophrenic post-mortem tissue by [^{3}H]NGD-94-1 binding. Prior to in vivo preclinical studies, it was established that L-745870 had high affinity for the rat D_4 receptor (rD_4 K_i 1.5 nM, 1000-fold selective over rD_2) and selectivity over a wide range of other neurotransmitter receptors. Importantly, L-745870 was inactive at Na^+ channels, very weak (IC_{50} 9 μM) in the Diltiazam (Ca^{2+} channel) assay, and weak (EC_{25} 2.5 μM) in a ferret papillary muscle functional assay of IKr activity. Modest affinity was detected for the σ binding site (K_i 130 nM) and 5-HT_2 receptors (K_i 200 nM), although hD_4 receptor selectivity was still >300-fold.

Several D_4 receptor antagonists have been examined in rodent tests predictive of antipsychotic activity and side effect liabilities (see section 4 for a discussion), and their behavioral profiles are summarized in Table 2. The first, overwhelming feature to note is that D_4 receptor antagonists have a benign side effect profile: they do not stimulate prolactin secretion, are not active in tests predicting extrapyramidal side effects, and only impair motor performance at relatively high doses. In contrast, these agents do appear to exhibit antipsychotic potential, and most will attenuate amphetamine and/or apomorphine-induced hyperactivity and reduce the disruption in prepulse inhibition induced by apomorphine in the rat. Studies with L-745870, however, seriously question the role of the D_4 receptor in mediating these "antipsychotic-like" effects. In the absence of neurochemical or behavioral responses known to be unambiguously mediated through D_4 receptor activation, surrogate assays were used as an alternative means of estimating doses *selectively* occupying D_4 receptors in vivo. First, L-745870 was shown to dose-dependently displace in vivo [^{3}H]-SKF 10,047 (**48**)



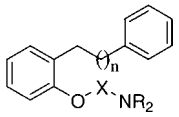
48
SKF 10047

binding to the σ recognition site (ED_{50} = 3 mg/kg, p.o.) and, based on the fact that the affinity of L-745870 is 87-fold greater at the rat D_4 receptor, it was predicted that 35 μg/kg L-745870 would occupy 50% of D_4 receptors in vivo. Extrapolations from the σ binding assay also predicted that, in rats, greater than 90% of brain D_4 receptors would be blocked at a dose of 1 mg/kg (p.o.) L-745870. Second it was shown that L-745870 dose-dependently inhibited mescaline-induced head twitches (a 5-HT_2 mediated effect) in rodents (ED_{50} = 7 mg/kg, p.o.) and, based on the relative affinities for the D_4 and 5-HT_2 receptor, it was predicted that a dose of 50 μg/kg would occupy D_4 receptors in vivo. Given that inhibition of amphetamine-induced hyperactivity is observed at doses clearly in excess of those maximally occupying D_4 receptors in rodent brain, it would appear that some other activity must explain these behavioral results. Likely explanations would include 5-HT_2 receptor antagonism (see below) or even D_2 receptor blockade since brain concentrations in excess of 10 μM have been measured following a dose of 3 mg/kg (p.o.) L-745870. Importantly, these results clearly demonstrate that, despite excellent receptor selectivity in vitro, L-745870 penetrates the brain sufficiently well that activity at other neurotransmitter receptors can be detected. While this unique approach of determining doses at which D_4 receptors are selectively occupied has only been applied to L-745870, it is possible that similar explanations may also account for the apparent "antipsychotic-like" effects of other D_4 receptor antagonists. In particular, it seems unlikely that D_4 receptor blockade is responsible for the ability of U-101,387 and CP-293019 to reverse apomorphine-induced disruptions in prepulse inhibition[161] since dopamine agonists still disrupt prepulse inhibition in D_4 receptor knock-out mice whereas the response is lost in D_2 receptor knock-out animals.[162]

To date L-745870 is the only selective dopamine D_4 receptor antagonist to have completed clinical trials in acutely psychotic inpatients.[163] In a randomized, double-blind study 12 patients received placebo and 26 patients received 15 mg of L-745870 daily, a dose which was predicted, based on the animal data described above and human pharmacokinetic data, to occupy greater than 90% of D_4 receptors in humans throughout the study. This clinical trial clearly showed that treatment with

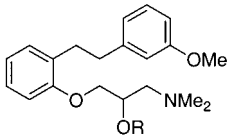
L-745870 for 4 weeks failed to improve psychotic symptoms; indeed more patients treated with L-745870 discontinued treatment because of insufficient therapeutic response, and those completing the study showed some worsening of symptoms compared to the placebo-treated group. While these observations should be viewed with caution because of the small number of patients, these results would suggest that selective blockade of dopamine D_4 receptors is not sufficient to alleviate the acute symptoms of schizophrenia. Clinical assessment of other D_4 receptor antagonists will be important in confirming these observations.

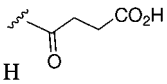
b. 5-HT$_{2A}$ Receptor Antagonists. The 5-HT$_2$ receptor is another receptor for which clozapine has high affinity that has been singled out as a specific target for the treatment of schizophrenia. In particular, while the 5-HT$_2$ receptor family comprises three receptor subtypes (5HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors), interest has focused on the 5-HT$_{2A}$ receptor because of its discrete localization in key brain areas.[164] There are a number of different classes of compounds with high affinity for 5-HT$_{2A}$ receptors. One of these series is the ω-phenylalkylphenoxyalkylamines[165] with a general structure described as in **49**. The 5-HT$_2$ receptor is



49

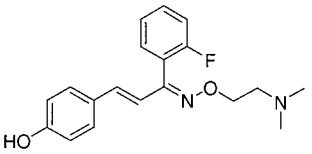
tolerant of a number of changes to both the length of the chain between the two benzene rings and the nature of the side chain bearing the basic nitrogen; however, the D_2 affinity does depend on these factors, and one is therefore able to obtain either selective or nonselective ligands from this series. A particular example of a 5-HT$_2$ receptor selective ligand is sarpogrelate[166] (MCI 9042, **50**) and its metabolite (**51**). Compound **50** was developed as an inhibitor of platelet aggregation, but **51** is also a high affinity 5-HT$_2$ ligand with activity in CNS assays. The affinity at 5-HT$_2$ receptors is not stereospecific, and



50 R = 

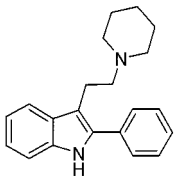
51 R = H

the compound is not selective for h5-HT$_{2A}$ over h5-HT$_{2C}$. Perhaps structurally related to this is the Sanofi oxime SR46349[167] (**52**) which has a similar "triangular" arrangement of two benzene rings and a nitrogen atom: in the former, compounds linked with a chain from one of the benzene rings; and in the latter, coming off the linker between the two rings. Compound **52** has 70-fold binding selectivity[168] for h5-HT$_{2A}$ over h5-HT$_{2C}$ receptors. A further example of compounds having this triangular pharmacophore are a series of 2-arytryptamines from Merck[169] exemplified by **53**, a nanomolar h5-HT$_{2A}$ receptor antagonist with 100-fold binding selectivity over h5-HT$_{2C}$ receptors.



52

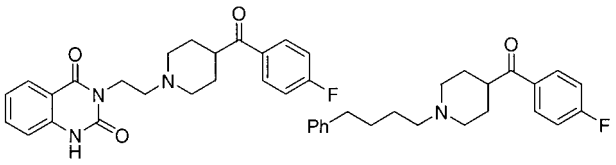
SR46349



53

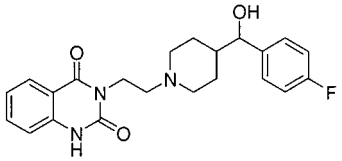
Fananserin (RP62203, **12**) was originally described[170] by Rhone-Poulenc Rorer as a high affinity (K_i 0.26 nM) rat 5HT$_2$ receptor antagonist with low affinity (K_i >1000 nM) at D_2 receptors and selectivity over α_1 adrenoreceptors (K_i 38 nM). The series is described as being "incidentally discovered" and structurally unrelated to ritanserin. Changes were made to the naphthosultam portion of the molecule: the 4-fluorophenylpiperazine was replaced with piperidines, tetrahydropyridines, and heterocyclic replacements for the benzene ring. The three-carbon chain length is preferred. Fananserin was chosen as the optimal compound for selectivity and in vivo properties, giving a potent and long lasting block of mescaline-induced head twitch in rat (ED_{50} 0.99 mg/kg p.o. at 1 h, 2.4 mg/kg p.o. at 6 h). Subsequently it has been reported that fanaserin has high affinity (K_i 2.9 nM) at hD_4 dopamine receptors.[171] In our hands,[168] fananserin has very high affinity (K_i 0.04 nM) at human cloned 5HT$_{2A}$ receptors, and some selectivity (30-fold) over h5HT$_{2C}$.

Some other structural series of 5-HT$_{2A}$ ligands fall into a "linear" pharmacophore class. The prototypical 5HT$_2$ receptor antagonist is ketanserin (**54**) with high 5HT$_{2A}$ receptor affinity and moderate selectivity over 5-HT$_{2C}$ receptors. There are a great many of this type of structure with affinities at 5-HT$_2$ and D_2 receptors, many of which use a benzofused heterocyle (benzisoxazole or benzthiazole) as a replacement for the benzoyl group of ketanserin, and are well covered in ref 172. Examples include risperidone and sertindole for which there is a published model[173] for predicting antagonist selectivity for 5HT$_2$ over D_2. Structure−activity relationship studies have been published on analogues of ketanserin (**54**), in which it has been shown[174] that the quinazolinedione moiety can be dramatically simplified to give the phenybutyl analogue (**55**) with only a small reduction in 5HT$_{2A}$ receptor affinity (K_i 5.3 nM vs 3.5 nM for **54**). Interestingly, for **54** the carbonyl of the benzoyl group is important, with reduction to **56** leading to a 100-fold loss in affinity. A further study[175] by the same group has shown that that the importance of the carbonyl group depends on the nature of the chain on the basic nitrogen. The analogue with a phenethyl group is more tolerant of reduction of the benzoyl group, with the affinity increasing 4-fold from **57** (K_i 9.6 nM) to **58** (K_i 2.5 nM), than that with a phenbutyl group, in which there is a 26-fold in loss in affinity going from **55** (K_i 5.3 nM) to **59** (K_i 126 nM). Compound **58** (MDL11939) was originally developed by Hoechst Marion Roussel as a class III antiarrhythmic.[176] The compound was also tested in humans[177] as a potential anxiolytic, but while



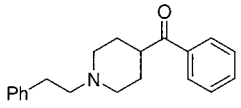
54
Ketanserin

55



56

it showed no adverse effects it was not efficacious for this use. Hoechst Marion Roussel went on to substitute both of the aromatic rings of this molecule and resolve the molecule, leading to M 100907 (**11**).[44]



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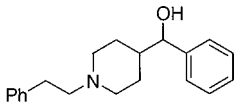
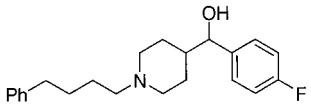
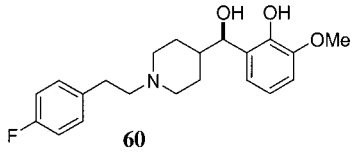


58
MDL 11939



59

M 100907 is a high affinity, selective antagonist at human and rat $5HT_{2A}$ receptors (K_i 1.5 and 1.4 nM, respectively) with 2 orders of magnitude binding selectivity over $5HT_{2C}$ and rat α_1 adrenoceptors, and 2600-fold binding selectivity over hD_2 receptors. It has a low affinity[168] at the IKr channel (K_i 1060 nM). M 100907 has been described[178] as being well absorbed but extensively first pass metabolized in rats and dogs, the major phase I metabolite being the product of O-demethylation (**60**). The doses used in behavioral experiments give an



60

indication of the oral bioavailability: in mice the ED_{50}'s for i.p. and p.o. antagonism of 5-MeODMT induced head twitches were 0.03 and 2.8 mg/kg, respectively, and the corresponding ED_{50}'s for antagonizing amphetamine induced hyperlocomotion in mice were 0.08 and 0.62 mg/kg. Thus, the difference between oral and i.p. doses in behavioral experiments in mice were 1 to 2 orders of magnitude. In our hands,[179] the compound has oral bioavailability of <3% in both rats and rhesus monkeys. Nonetheless, Hoechst Marion Roussel have progressed the compound to phase III clinical trials for schizophrenia in humans. A study in rats[178] using in vivo microdialysis has shown that M 100907 has better brain penetration (brain/plasma AUC approximately 1 after 50 mg/kg p.o.) than its metabolite (**60** brain/plasma AUC approximately <0.13 after 50 mg/kg p.o. of M 100907), and that the metabolism does not take place in the brain. The area under curve data (AUC) measured in these experiments also support low rat oral bioavailability, with the plasma AUC following 50 mg/kg p.o. dosing being some 10-fold lower than that following 5 mg/kg iv.

M 100907[44] exhibits an antipsychotic-like profile in rodent behavioral/neurochemical tests. Thus M 100907 was reported to attenuate (i) PCP-induced hyperactivity in mice[180] and PCP-induced increases in nucleus accumbens dopamine efflux in rat,[181] (ii) 3,4-methylenedioxymethamphetamine (MDMA)-induced dopamine release[182] and hyperactivity[183] in rat, (iii) hyperactivity induced by amphetamine in mice[184] and (iv) the deficits in prepulse inhibition induced by MK-801.[185] In contrast, M 100907 failed to induce catalepsy or block stereotyped behaviors, suggesting that the compound has antipsychotic potential with negligible EPS liability. On the basis of this encouraging preclinical profile, the compound was taken forward into clinical trials. PET studies[186] in humans using doses of 10 and 20 mg of M 100907 to displace [^{11}C]-spiperone from cortical $5HT_2$ receptors have shown that following both doses central $5HT_2$ receptors are occupied to an extent of 70−90% 8 h post dose and that the receptor occupancy is reduced by about 20% at 24 h in the 10 mg group but is maintained at essentially full occupancy in the 20 mg group. The plasma half-life of M 100907 in humans is 6.6 h, but the receptor occupancy does not seem to follow this half-life. Possible explanations are that the compound is slow to come off the receptor or out of the brain or that for a considerable portion of time the receptors are saturated, leading to the apparent plateau of peak occupancy. The results of this study, however, are conclusive. Good receptor occupancy is achieved in humans with this compound at very low plasma levels (>75% occupancy at 1−3 ng/mL) and that single daily dosing of 20 mg of M 100907 leads to an essentially complete blockade of $5HT_2$ receptors for the duration of the experiment. Another PET study[187] in two schizophrenic patients using [^{11}C]-M100907 confirmed these findings. Using these data, clinical trials have been conducted in schizophrenic patients. A phase II clinical trial[188] in 47 patients using doses of 10, 20, or 40 mg/day over a 6 week period showed, with the combined treatment groups, a significant improvement in BPRS scores compared to the placebo group, with no increases in EPS. However, the development of M 100907 for acute schizophrenia has been discontinued following results of large (1000 patient) phase III clinical trials.[189]

Fananserin has also been tested[190] in a phase II clinical trial for the treatment of schizophrenia. Patients were dosed up to 250 mg bid for 4 weeks, the dose being chosen as the highest one consistent with safety, and gave trough plasma levels of at least 70 ng/mL. While receptor occupancy was not measured in humans, extrapolations from the animal data[191] suggest that this dose is likely to be sufficient to block both hD_4 and $h5HT_{2A}$ receptors in the patients. The clear outcome of this trial was that fananserin had no effect on either the positive or negative symptoms of schizophrenia.

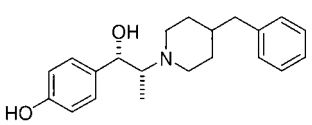
To summarize, the past decade has seen a large effort

aimed at mimicking the effect of clozapine by selectively blocking a single receptor in the CNS of schizophrenic patients. In clinical trials, blocking either dopamine D_4 receptors or 5-HT_{2A} receptors or both has shown no improvement in the symptoms of the disease. While there remain many new receptors (e.g., 5-HT_6, 5-HT_7[192]) at which clozapine has high affinity, and there will undoubtedly be more discovered during the process of mapping the human genome, experience thus far would suggest that this is not a fruitful approach to the development of novel antipsychotics.

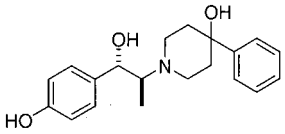
7. Atypical Antipsychotics: Potential Novel Approaches

a. Glutamate. As discussed in section 3c, there is growing interest in the potential role of the excitatory amino acid system in schizophrenia. The observed effects of noncompetitive NMDA receptor antagonists in humans and reduction of glutamate function in schizophrenic post-mortem tissue has prompted speculation that pharmacologically enhancing NMDA receptor function may be beneficial in treating aspects of the disorder. However, while fewer studies have been conducted, largely due to the lack of suitable compounds, it may be feasible to enhance glutamate transmission via activation of metabotropic or α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) receptor subtypes.

a. i. *N*-Methyl-D-aspartate (NMDA) Receptors. NMDA receptors are comprised of multiple subunit combinations, the exact stoichiometry of which is unknown in native receptors. At least three NMDA receptor subunits, each a separate gene product, have been identified: NR1, of which there are eight isoforms (a−h), NR2 of which there are four subtypes (A−D), and NR3A (formerly termed NMDAR-L or χ-1)[193,194] which is developmentally regulated but is present in some regions of adult rat brain. The NR1 subunit appears to be an essential requirement for the formation of functional NMDA receptors which are formed by the combination of at least one obligatory NR1 subunit with at least one other subunit from a separate gene (NR2 A−D) or NR3A. It is conceivable that NMDA receptors exist both as doublets (e.g., NR1/NR2) and/or triplets (for example NR1/NR2x/NR2y or NR1/NR2/NR3A) in native tissue, making the number of potential receptor variants extremely large. An extra level of complexity, which may be exploited pharmacologically, is that NMDA receptor subunit combinations contain multiple binding sites that allosterically regulate receptor function. Thus, point mutation and electrophysiological studies have demonstrated that the glycine modulatory site is located within the NR1 subunit and may therefore be common to all NMDA receptor variants.[195] In contrast, the ifenprodil (**61**) binding site appears to be associated specifically with amino acid residues where the NR2B subunit overlaps with the NR1 subunit.[196,197] Consequently, ifenprodil has little affinity for other NMDA subunits, and its action is to selectively block NR2B containing NMDA receptors. As NMDA receptor subunit combinations are differentially distributed in the CNS, it is conceivable that these receptors are involved in many CNS processes. Pharmacologically, this is a relatively unexplored area and the challenge here is to develop subtype selective drugs that modulate glutamate receptor function without causing possible mechanism related side effects such as excitotoxicity, decreased seizure threshold, ataxia, and cognitive impairment. To date, the only available subtype selective NMDA receptor ligands that have been identified are ifenprodil and its analogues, for example CP101606 (**62**), which are antagonists at NR2B containing receptors and as such would not be expected to have antipsychotic activity.

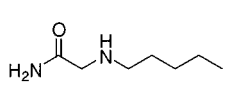


61
Ifenprodil

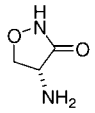


62
CP 101606

Uniquely, the NMDA receptor requires the presence of both glutamate and glycine to be activated. As high concentrations of glutamate are neurotoxic, attempts have been made in a number of open label clinical trials to increase glutaminergic transmission by administration[198−200] of high doses of glycine, glycine pro-drugs,[201] for example milacemide (**63**), or amino acid analogues with affinity for the NMDA/glycine site, for example D-cycloserine (**64**),[202] with or without concomitant neuroleptic therapy. The results of these trials have been variable and somewhat unconvincing, with some studies showing clinical improvement albeit largely with regard to negative symptoms, and others showing either no benefit or worsening of symptoms (see ref 203 for review). There are two problems with such studies. First, it is possible (though unlikely[204]) that the glycine/NMDA site is saturated with endogenous glycine under normal physiological conditions in which case it would be difficult to enhance glutamate transmission via activating this site. Second, these studies may be compromised by the inadequacy of the compounds used in the clinical trials. Glycine has poor brain penetration, and systemic administration is consequently a relatively inefficient method of increasing brain glycine concentration. Milacemide readily crosses the blood brain barrier where it is converted to glycine and glycinamide, but it also has additional pharmacology that may relate to monoamine oxidase A inhibition[204] and which may complicate its therapeutic effects. D-Cycloserine is a partial agonist at the glycine/NMDA site and may act as an agonist or antagonist depending on the concentration of glycine at the NMDA receptor. If under such conditions D-cycloserine behaved as an antagonist at the NMDA/glycine site, psychotomimetic effects may be anticipated.



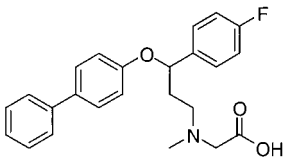
63
Milacemide



64
D-Cycloserine

Therefore, to fully test this hypothesis it is essential that a more effective method of enhancing NMDA transmission is developed. One such approach may be to increase the availability of glycine in the CNS by

inhibiting its reuptake. Two glycine transporters, GLYT-1 and GLYT-2, have recently been cloned.[205−207] Each of these is predicted to contain 12 hydrophobic membrane spanning regions and are dependent on Na^+/Cl^-, placing them in the monoamine neurotransmitter transporter family. They are differentially distributed in the mammalian CNS: GLYT-1 is expressed in most brain regions including the hippocampus, where it is found in close proximity to NMDA receptors, whereas GLYT-2 sites are predominantly located in brainstem and spinal cord.[208] In support of the suggestion that blockade of the GLYT-1 transporter would increase NMDA receptor mediated transmission, a recent study demonstrated that local application of *N*-[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine (**65**), a selective and potent GLYT-1 reuptake inhibitor, enhanced NMDA mediated currents in rat hippocampal slices in vitro.[209] Furthermore, preclinical evidence that inhibition of GLYT-1 transport may have antipsychotic activity was provided by studies with glycyldodecylamide. This is a relatively weak GLYT-1 inhibitor, but it is brain penetrant and was shown to block the uptake of glycine in rat cortex and the hyperactivity induced by PCP in rats.[210,211] Further studies are required with more potent compounds, for example ORG 24598 (**66**),[212] to fully characterize this novel pharmacological approach. However, it is also worth remembering that even the most positive clinical trials with glycine replacement therapy only improved negative symptoms, and it is quite possible that this treatment strategy will not address all clinical manifestations of the disorder.



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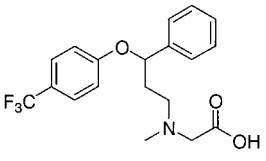
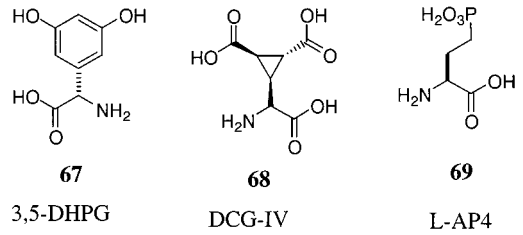


66

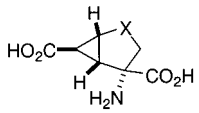
Org 24598

a. ii. Metabotropic (mGlu) Receptors. Metabotropic glutamate (mGlu) receptors, of which there are eight subtypes ($mGlu_{1-8}$), are categorized into three groups according to their agonist pharmacology, sequence similarity, and signal transduction pathways. Group I mGlu receptors consist of $mGlu_1$ and $mGlu_5$, both of which are positively coupled to phospholipase C and are activated by (*S*)-3,5-dihydroxyphenylglycine (**67**, 3,5-DHPG). Group II mGlu receptors include the $mGlu_2$ and $mGlu_3$ subtypes, which are negatively coupled to adenylyl cyclase and are preferentially activated by (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (**68**, DCG-IV). Group III mGlu receptors consist of $mGlu_{4/6/7/8}$ and are also negatively coupled to adenylyl cyclase but are preferentially activated by (*S*)-2-amino-4-phosphonobutyric acid (**69**, L-AP4).

With respect to the relevance of these receptors to schizophrenia, several studies have reverted to the dopamine hypothesis of schizophrenia and focused on the possible role of metabotropic glutamate receptors in the regulation of mesolimbic dopamine function. In situ hybridization and pharmacological studies demonstrated that not only are $mGlu_{1-5}$ receptors located in basal ganglia structures including the nucleus accumbens,[213] but also that mGlu receptor ligands modulate dopamine neuronal function in these structures.[214−217] However, the ligands used in such studies are not subtype specific, particularly when used in high concentration, and further analysis of the involvement of individual subtypes await the development of more selective ligands. The group II mGlu receptors have recently become of considerable interest following the discovery of LY354740 (**70**) and LY379268 (**71**) which



67 3,5-DHPG **68** DCG-IV **69** L-AP4

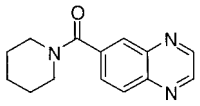


70 X = CH_2 LY 354740
71 X = O LY 379268

are potent, brain penetrant, selective group II mGlu receptor agonists.[218,219] Functional studies with these compounds suggest that $mGlu_{2/3}$ receptors are located presynaptically on glutamate terminals where they may act as autoreceptors regulating glutamate release in vivo.[220,221] Immunocytochemical mapping studies have indicated that $mGlu_{2/3}$ receptors are located in several forebrain structures including the cortex and hippocampus both post- and presynaptically.[222] Recent behavioral and neurochemical studies with group II receptor agonists demonstrated an atypical antipsychotic-like profile with a novel mechanism of action independent of modulating dopamine release.[218,223] Thus LY354740 and LY379268 attenuated PCP induced increased locomotion in rats without significantly impairing motor performance or working memory in a delayed alternation test. Interestingly, and in contrast to haloperidol or clozapine, the $mGlu_{2/3}$ receptor agonists did not influence the hyperlocomotor effects of amphetamine although rearing induced by amphetamine was significantly attenuated by both compounds. The lack of effect of LY354740 on basal or PCP induced dopamine release in the nucleus accumbens or prefrontal cortex suggested a dopamine independent mechanism of action, which at present remains unclear. In keeping with the suggestion that group II receptor agonists activate terminal autoreceptors to decrease evoked release of glutamate in vitro,[221,224] LY354740 significantly blocked PCP induced increase of glutamate release in the nucleus accumbens and prefrontal cortex in vivo without affecting basal glutamate release per se. This suggests either that increased dopamine release is not required for the expression of PCP related behavior or that increased release of glutamate occurs after increased dopamine release. However, while the results of these studies seem promising, the lack of efficacy of LY354740 in attenuating PCP induced disruption of prepulse inhibition of the acoustic startle response[225] does not support

the utility of $mGlu_{2/3}$ receptor agonists as a novel class of atypical antipsychotic, and it is clear that further work is required to substantiate this claim.

a. iii. α-Amino-3-hydroxy-5-methyl-4-isoxazole proprionic Acid (AMPA) Receptors. Molecular cloning studies have shown that AMPA receptors are comprised of multiple subunits termed GluR1−4, each encoded by a separate gene and each occurring in alternatively spliced "flip" and "flop" forms that display different pharmacological properties. AMPA receptor subunits are widely expressed in virtually all regions of the mammalian CNS and appear to exist both in neurons and glia. Due to their involvement in mediating excitatory synaptic currents and their ability to flux calcium ions, it has been proposed that AMPA receptors are involved in synaptic plasticity and neuronal development and that they may contribute to excitotoxic and neurodegenerative mechanisms.[226] The pharmacology of these receptors is rather limited due largely to the relative paucity of ligands with selectivity over kainate and NMDA receptors. The positive allosteric modulators such as the ampakines, as typified by CX516 (**72**), are

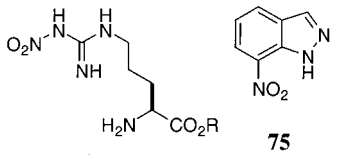


72

CX516

a class of brain penetrant molecules that selectively activate AMPA receptors and were initially developed as cognitive enhancers. It is conceivable therefore that, as with glycine replacement therapy, such compounds could also enhance glutamate transmission in schizophrenia. Recent preclinical studies have shown, however, that while **72** was ineffective in reducing methamphetamine induced rearing behavior in rats when given alone, it significantly attenuated this behavior when given in combination with a subthreshold dose of either a typical or atypical antipsychotic.[227] Such findings, while limited, are of interest, although it would suggest that such compounds are only likely to be used as add-on therapy with established antipsychotic agents. It is unclear, at present, if this would represent a significant advantage over conventional treatment.

b. Nitric Oxide (NO). Nitric oxide is an important inter- and intracellular messenger in many peripheral tissues and the CNS. NO is formed by the enzyme nitric oxide synthase (NOS), which converts arginine into NO and citrulline in response to increased intracellular calcium levels following, for example, activation of the NMDA receptor. NO is a diffusable gas, and one potential molecular target appears to be soluble, guanylyl cyclase, in which it binds to the haem moiety, activating the enzyme and increasing the synthesis of cyclic GMP. Post-mortem studies of schizophrenic brain tissue have shown an increase in the number of NOS positive neurons in the pedunculopontine nucleus, a region involved with sensory gating, and also in the concentration of NOS in the cerebellum.[228−230] Methylene blue, a compound that inhibits NOS and soluble guanylyl cyclase activity,[231] moderately improved symptoms in schizophrenic patients who were refractory to conventional neuroleptics.[232] In keeping with these findings, animal studies showed that inhibition of NOS activity with methylene blue, L-NOARG (**73**), L-NAME (**74**), or 7-nitroindazole (**75**) attenuated the hyperactivity



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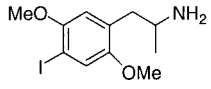


76

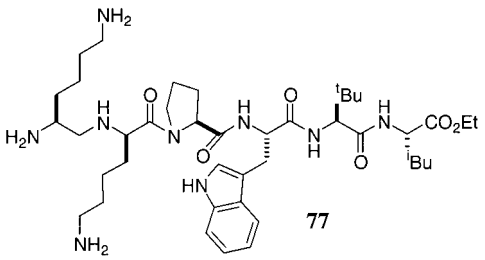
DOI

73 R = H L-NOARG
74 R = Me L-NAME

and disruption of prepulse inhibition by noncompetitive NMDA receptor antagonists MK-801 and PCP but not amphetamine.[233−238] When given alone, these inhibitors did not affect startle responses or baseline locomotor activity, neither did they induce catalepsy. Furthermore, it has recently been shown that head twitch behavior induced by the hallucinogenic $5\text{-}HT_2$ receptor agonist DOI (**76**) was prevented by 7-nitroindazole and L-NAME.[239] While these findings are exciting, unfortunately all currently available NOS inhibitors induce unacceptable side effects including hypertension and cognitive impairment that may be due to poor selectivity for different NOS isoforms. It will be necessary to find compounds with improved selectivity for neuronal NOS before this issue can be addressed.

c. Peptides. c. i. Neurotensin (NT). Neurotensin is a 13 amino acid peptide produced by cleavage of a larger precursor protein and is located in peripheral tissues, predominantly the gastrointestinal tract and in the brain, where it is found in close proximity to mesolimbic and nigrostriatal dopamine neurons.[240] Two receptor subtypes, NT_1 and NT_2, have recently been cloned and are expressed in rodent and human brain.[241−244] The physiological significance of the NT_2 receptor remains obscure as it appears to have a lower affinity for NT than the NT_1 receptor. Furthermore, studies in vitro using cloned human receptors demonstrate that NT behaves as an agonist at NT_1 and an antagonist at NT_2 receptors.[245] Such findings raise the question of whether the NT_2 receptor can be considered a true NT receptor, and comparable studies at native receptors will be required to confirm this unusual in vitro profile.

NT has been suggested,[246] on the basis of co-localization and behavioral studies, to regulate the function of mesolimbic dopamine neurons in the CNS. This is supported by more recent studies showing that central administration of NT or systemic administration of metabolically stable NT analogues, e.g., NT_1 (N^{α}Me-Arg-Lys-Pro-Trp-Tle-Leu) or PD 149163 (**77**) blocked am-



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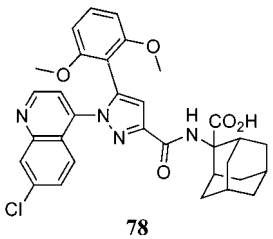
phetamine, PCP, or MK-801 induced locomotor activity

and prepulse inhibition[247−249] without inducing catalepsy or affecting baseline startle responses. This profile is certainly consistent with that of an atypical antipsychotic although effects on prolactin secretion appear not to have been determined. There are potential problems with the use of NT1 receptor agonists, as NT is known to induce widespread autonomic side effects including gastric hypomotility, gastric acid hyposecretion, hypotension, and hypothermia.[250] Furthermore, many of the physiological, neurochemical, and electrophysiological effects[251] of NT show rapid desensitization, and while this may serve to reduce side effect liabilities, it may also diminish the antipsychotic effects of NT_1 agonists following repeated dosing. While there are no published clinical trials with selective NT_1 receptor agonists, Sanofi have recently taken SR 48692 (**78**), a non-

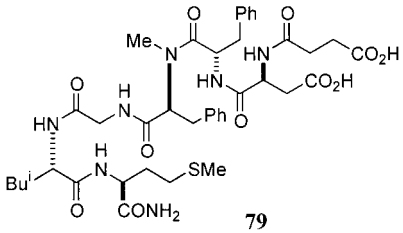


78

SR 48692

peptide, high affinity NT_1 receptor antagonist with agonist efficacy at NT_2 receptors, into phase II trials.[252] Acute administration of SR 48692 was found to have no effect on spontaneous locomotion or basal release of dopamine in the nucleus accumbens[253,254] and potentiated the locomotor effects of subthreshold doses of methamphetamine.[255] While the latter effect is not consistent with that of an antipsychotic agent, repeated treatment with SR 48692 for 15 days was found to decrease dopamine release in the nucleus accumbens.[256] The latter findings suggest that long-term treatment with such compounds could be beneficial in the treatment of schizophrenia or other psychoses. However, it is worth remembering that this particular compound is both an antagonist and agonist at NT_1 and NT_2 receptors, respectively,[245] and given the complexities of central neurotensin-dopamine interactions it is difficult to assess whether the in vivo profile of this compound is a consequence of blocking NT_1 or activating NT_2 receptors.

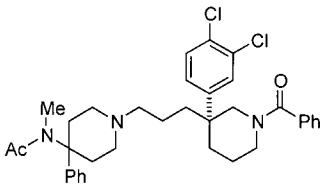
c. ii. Tachykinin Receptor Antagonists. The mammalian tachykinins, which include the undecapeptide substance P, neurokinin A, neurokinin B, and neuropeptide K, are a family of closely related peptides encoded by two preprotachykinin genes (for review, see ref 257). They are present in mammalian brain tissue, and their biological effects are mediated by three tachykinin receptor subtypes ($NK_{1,2,3}$) which are preferentially selective for substance P, neurokinin A, and neurokinin B, respectively. NK_3 receptors, visualized autoradiographically using the selective agonist [^{3}H]-senktide (**79**), are widely distributed in rodent CNS with moderate density in cortex, substantia nigra, basolateral amygdala, caudate putamen, nucleus of the diagonal band, ventral tegmental area, and median raphe nucleus.[258] Interestingly, NK_3 receptors appear to have an overlapping distribution with tyrosine hydroxylase containing neurons in rat mesencephalic dopamine cell body areas A8, A9, and A10[259] consistent with the suggestion that NK_3 receptors may regulate midbrain dopamine neuronal activity.[260−263]



79

Senktide

However, NK_3 receptors appear to be absent[264] or very weakly expressed in normal human brain tissue, possibly with a different cellular and anatomical distribution to that observed in the rat.[265] Nevertheless, studies of NK_3 receptor density in schizophrenic brain tissue have not yet been reported, and it is conceivable that NK_3 receptor density is altered by the disease process. Despite the controversial evidence for the presence of NK_3 receptors in human brain, Sanofi have recently taken[252] a potent non-peptide NK_3 antagonist, SR 142801 (**80**),[266] into phase II clinical trials for schizo-

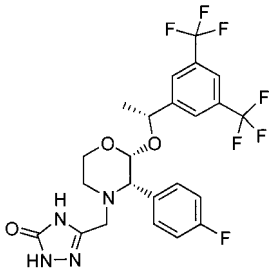


80

SR 142801

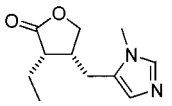
phrenia, the outcome of which is awaited with interest. It is less clear that substance P acting directly at NK_1 receptors can modulate the function of rodent midbrain dopamine neurons. Thus while NK_1 receptors appear to be present on dopamine cell bodies,[267] and infusion of a selective NK_1 receptor agonist into the ventral tegmental area increased dopamine metabolism in prefrontal cortex and nucleus accumbens,[268] selective NK_1 receptor agonists were also found to be less effective than NK_3 preferring agonists in causing dopamine mediated behavior.[261] These apparently contradictory findings may reflect the inadequate research tools available at the time. NK_1 receptors appear to be the predominant subtype in human brain and are found in high to moderate density in the striatum, cortex, hippocampus, and importantly in the ventral tegmental area.[269] However, the selective, potent NK_1 receptor antagonist MK-869 (**81**)[270] was recently found to lack efficacy in a four week placebo and haloperidol controlled clinical trial in schizophrenic in patients,[271] demonstrating that this is not a viable novel approach to the treatment of schizophrenia.

d. Cholinergic Transmission. d. i. Muscarinic Receptors. Muscarinic receptor antagonists have been used for many years to limit the severity of EPS induced by chronic neuroleptic medication, by functionally antagonizing the effects of dopamine blockade. It has been

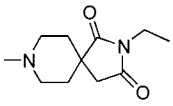


81
MK 869

suggested that impairment of the cholinergic system in schizophrenia may induce hyperfunction of dopamine neurons. Several atypical antipsychotics including clozapine and olanzapine have moderate affinity and are partial agonists for cholinergic M_1, M_2, and M_4 receptors[272] (also see ref 42 and Table 1). This activity may contribute to their reduced EPS liability and possibly their clinical efficacy. Interestingly, recent studies have demonstrated that several nonselective muscarinic receptor agonists including pilocarpine (**82**), RS 86 (**83**),[273] and PTAC (**84**),[274] a potent, selective, partial agonist at



82
Pilocarpine



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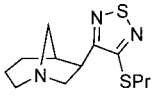
RS 86



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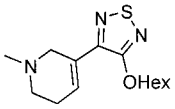
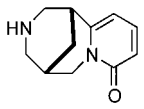
PTAC



85
Xanomelline

M_2 and M_4 receptors and an antagonist at M_1, M_3, and M_5 receptors, displayed antipsychotic activity in animal assays. PTAC inhibited ventral tegmental dopamine cell firing and blocked conditioned avoidance responding, and apomorphine induced climbing without inducing catalepsy, salivation, or tremor, although spontaneous locomotor activity was markedly reduced. Consistent with this behavioral profile, xanomeline (**85**), a functionally selective M_1/M_4 receptor agonist, was found, in a clinical trial for Alzheimer's disease, to improve psychotic symptoms including delusions, hallucinations, agitation, and fearfulness in 9 out of 17 patients although some cholinergic adverse events were reported.[275] This clinical observation suggests that M_1/M_4 receptor agonists may represent a novel approach to antipsychotic therapy that acts possibly by functionally opposing the action of dopamine in the mesocorticolimbic system. The difficulty with this class of compound is ensuring adequate selectivity over M_2/M_3 receptors where agonist activity may be associated with undesirable, peripherally mediated side effects.

d. ii. Nicotinic Receptors. There is some evidence for a reduction of nicotinic cholinergic function in schizophrenia. Thus the density of nicotinic receptor subtypes labeled by [^{3}H]-cytisine (**86**) and [^{125}I]α-bungarotoxin are significantly decreased in the dentate and CA3 regions of schizophrenic brain,[276] although a reduction of these sites could be due to other factors including smoking and antipsychotic/anticholinergic medication. Schizophrenic subjects show clear deficits in the gating



86
Cytisine

of auditory evoked stimuli, and nicotine administration, either in the form of gum or from smoking cigarettes, was found to transiently normalize these deficits possibly by activating the α_7 subtype.[277,278] Consistent with this, a similar failure to suppress auditory responses is also found in rats after blockade of α_7-nicotinic receptors in the hippocampus.[279] Additionally, nicotine can reverse some of the cognitive deficits, including those of attention and memory, observed in schizophrenics following administration of dopamine D_2 receptor antagonists.[280] However, nicotinic receptors including the α_7 subtype[281] show rapid desensitization following activation by agonists and it is therefore difficult to determine if the observed effects of nicotine administration are due to receptor activation or desensitization.

Conclusions

Schizophrenia is a severe, common, and poorly understood disease with a strong genetic component, but no susceptibility or causative genes have been discovered to date. Dopamine D_2 receptor antagonists provide partially efficacious symptomatic treatment but suffer from multiple adverse effects. Clozapine was the first of the so-called atypical antipsychotics, found to overcome the adverse effects observed with D_2 receptor antagonists, and in patients was established to be more efficacious against positive and negative symptoms. The reasons for the superior clinical effectiveness of clozapine, which has high affinity for multiple receptors, are not understood. Although a number of compounds which overcome some of the limitations of the dopamine D_2 receptor antagonists have recently reached the market, clinical experience with these new agents is limited. It may be some time before their efficacy against all aspects of this disorder is determined, and there are still a number of side effect issues with some of these drugs, due to their relatively high affinity for multiple receptors. It is also clear that studies thus far with compounds selective for a single receptor targeted by clozapine, for example fananserin, M 100907, and L-745870 which are selective for 5-HT_{2A} or D_4 receptors, have failed to show comparable clinical efficacy. Thus, it seems unlikely that activity at a single receptor will convey clozapine's atypical antipsychotic profile. What is less obvious is how the next generation of antipsychotics will be developed, as the fundamental lack of understanding of the disease process obviates a rational therapeutic strategy based on intervention or palliative therapy. A more long-term approach may be to exploit information from clinical functional imaging studies which are becoming increasingly sophisticated, provid-

ing more detailed information of the brain structures, pathways, and circuits involved in various aspects of the disorder. Finally, advances in genomics including gene chip technology, proteomics,[282] and transgenic animal technology[99,100,102,283] may provide novel molecular targets and more appropriate animal models that will ultimately lead to a greater understanding of schizophrenia and hence improved treatment.

Biographies

Michael Rowley was educated in Cambridge, doing a Ph.D. with Ian Fleming, before 2 years postdoctoral work with Yoshito Kishi at Harvard. He joined Merck in 1988. His interests have been in the areas of ion channels and G-protein coupled receptors in the central nervous system. He has recently moved to become the Head of Medicinal Chemistry at the Istituto di Ricerche di Biologia Molecolare (IRBM), the Merck Research Laboratory site in Rome, Italy.

Linda J. Bristow graduated from Somerville College, Oxford University, in 1984 with a B.A. honors degree in physiological sciences. She moved to the Physiology and Pharmacology Department at Nottingham University to work with Geoff Bennett and received her Ph.D. in 1988. She joined the Behavioural Pharmacology Department at Terlings Park in 1988 and recently took up the position of Laboratory Head, Psychopharmacology, at MRL, San Diego, CA.

Peter H. Hutson received his Ph.D. from the Department of Neurochemistry, Institute of Neurology, London. He joined Merck in 1989. His current interests are the neuropharmacology of schizophrenia and depression. He is Senior Investigator in the Department of Behavioural Neuroscience at Terlings Park.

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