The University of Glasgow.

# AN INVESTIGATION OF THE ANOMALOUS LOW INCIDENCE OF MOTOR SIDE EFFECTS OF THE NOVEL ANTIPSYCHOTIC DRUG – Y931.

A thesis submitted for the degree of Master of Science.

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**Dedication**

**To the memory of my mother and father**

**Sm. Sarbamangala Debi and Sri. Sudhir Chandra Mukherjee**

**And**

**To my family: My husband Mr.Sukumar Chatterjee and and my two sons Rana and Krishna, always encouraging me, giving me moral support.**

**To my two sisters: Sulekha and Subrota, for giving me courage and strength.**

## ABSTRACT

The quest for finding new antipsychotic drugs for schizophrenia, using clozapine as the model, with fewer side effects as compared to typical antipsychotic drugs, has produced quite a few new drugs in recent years. But none of these are completely free of extrapyramidal side effects (EPS), a condition that gets worse by prolonged use of antipsychotic drugs by some patients.

In recent years, it has been hypothesized that a lower affinity towards dopamine $D_2$ receptors and higher affinity for serotonin (especially at $5HT_{2C}$ receptors) for the new drugs may cause their atypicality and lower EPS.

Y931 is a new potential atypical antipsychotic drug (Morimoto et.al., 2000). It is also classed as a multi-affinity receptor target agent. It is suggested that Y931 gives very few (if any) extrapyramidal side effects (Morimoto et. el., 2002) in animal models at standard medical dosage. Y931 has a similar receptor profile to clozapine and olanzapine. But unlike these and some other antipsychotic drugs, Y931 has relatively high affinity for dopamine $D_2$ receptors. Thus some aspect of the pharmacology of Y931 could be different to clozapine and olanzapine and it may be working to suppress potential EPS generated by $D_2$ antagonism in some different way than these drugs.

Y931 has very high affinity for $5HT_{2C}$ receptors. There are significant levels of $5HT_{2C}$ receptor mRNA present in the terminal areas of the dopaminergic system, e.g., striatum and the nucleus accumbens. In vivo electrophysiological studies showed that the serotonergic system exerts an inhibitory action on the basal activity of midbrain DA-containing neurons. Thus it could be that there could be a functional relationship between serotonin and dopamine containing neurons, which could be either inhibition or facilitation of dopamine release. Several researchers showed that antagonism of $5HT_{2C}$ receptors can affect dopaminergic modulation (Bonhaus et al., 1997; Di Matteo et al., 1999; Kennett et al., 1997; Hutson et al., 2000; Wood et al., 2001).

The aim of this study is to investigate the characteristics and mechanisms of pharmacological activity of Y931 in relation to extrapyramidal side effects.

We used three different studies to analyse the compound Y931. Firstly, we studied the immediate early gene (IEG) induction profile of Y931 (10mg/kg, i.p.) together with haloperidol (1mg/kg, i.p.) and olanzapine (10mg/kg, i.p.) after 45 minutes following acute treatment. The level of expression of c-fos mRNA and zif268 mRNA was monitored in a number of brain areas related to motor control. We also monitored fra-2 mRNA in most of these areas. Results suggest that Y931 and olanzapine induced relatively less zif 268 mRNA expression in some layers of cortex as compared to haloperidol and/ or vehicle controls. In contrast, Y931 did not change c-fos mRNA expression in the motor cortex. In dorsal caudate putamen, Y931 increased zif 268 and c-fos mRNA expression. A similar effect was observed with haloperidol and olanzapine. This may reflect the high affinity of these drugs for $D_2$ receptors. In contrast to the results obtained with zif 268 and c-fos mRNA expression, no drug induced changes were observed in the level of fra-2 mRNA expression.

Secondly, we carried out a behavioural study on the effect of serotonin receptor antagonists using a $5HT_{2B/2C}$ receptor antagonist (SB200646, 10 mg/kg, i.p) and a selective $5HT_{2C}$ receptor (SB 242084) on haloperidol induced catalepsy. Y931 has very high affinity for serotonin receptors and it had been suggested that $5HT_{2C}$ receptor antagonism may attenuate haloperidol induced catalepsy (Curzon and Kennett 1990, Eberle-Wang et.al. 1996; Reavill et.al.1999). Thus the result would be able to clarify the possibility of whether Y931 might be using this mechanism to combat the EPS. For this, we treated animals with haloperidol alone and also pretreated different groups of animals with SB200646 or SB242084 (both at 10 mg/kg i.p.), 15 mins before haloperidol. Other groups of animals were treated with Y931 (10mg/kg i.p.) and olanzapine (10 mg/kg i.p.). The animals were then tested for catalepsy after different time points (up to 180mins), using a block test, a grid test and a bar test. We compared the results of combined treatment ($5HT_{2C}$ receptor antagonist + haloperidol) with Y931 and olanzapine treated animals. The result of this study was not conclusive but suggested that all drug

combinations may cause catalepsy-like effects in these tests, although in the case of Y931, there was difficulty in distinguishing catalepsy from sedation.

Thirdly, we studied the induction profile of some marker genes in the different regions of the basal ganglia, which may be involved in the process of EPS after acute treatment with antipsychotic drugs. After 45 mins drug treatment none of the marker gene mRNA expression levels were changed. The expression level of GAD67 mRNA was significantly reduced in the lateral globus pallidus after treatment with Y931 compared to controls and haloperidol after 180 mins treatment with drugs. For parvalbumin and grp75 (after 180 mins drug treatment), both mRNAs were expressed significantly higher than in control animals in the subthalamic nucleus after treatment with all three drugs. None of the marker genes showed altered expression of their mRNAs in the substantia nigra pars reticulata or substantia pars compacta. Furthermore, changes in the gene expression did not correlate with the degree of catalepsy detected.

Pretreatment with $5HT_{2C}$ receptor antagonists showed change in the effect of haloperidol in the globus pallidus. In the lateral globus pallidus, Y931 and olanzapine, but not haloperidol reduced GAD 67 mRNA expression level. However, pretreatment of haloperidol treated rats with the $5HT_{2C}$ receptor antagonist SB 242084 resulted in the reduction in GAD67 mRNA expression similar to that of olanzapine and Y931.

There was no change in the lateral globus pallidus after pretreatment with serotonin antagonists on the effect of haloperidol in the expression level of parvalbumin mRNA. In the subthalamic nucleus, pretreatment with the $5HT_{2C+B}$ receptor antagonist but not the $5HT_{2C}$ receptor antagonist, significantly lowered the effect of haloperidol upon parvalbumin mRNA levels. This result was also significantly lower than haloperidol.

In substantia nigra pars reticulata, the expression level of GAD67 mRNA and parvalbumin mRNA did not change.

Overall, the results suggest that Y931 tends to increase striato-pallidal activity and pallido-subthalamic activity. The contribution of $5HT_{2C}$ receptor activity is not clear. The higher affinity of Y931 for serotonin receptors ($5HT_{2C}$) did not seem to relate to the ability of Y931 to alter the activity of regions of the basal ganglia. The results suggest that Y931 affects striatal activity in a way predicted to lead to EPS. However, some action elsewhere in the basal ganglia may suppress this potential, although $5HT_{2C}$ receptors are unlikely to be involved.

## Acknowledgments

I would like to give my sincere thanks to Prof. B. Morris and Dr. J. Pratt for their enormous help and support to me to get through this project. The ancient 'Acharyya-Rishi'- [the great- learned men of ancient India] said, 'a teacher' is a good 'teacher' when he or she help their 'pupil' to be better than themselves. I think Prof. B. Morris and Dr. J. Pratt are good teachers. I could never have been able to do this work without their help.

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Thank you all.

The investigations presented in this thesis, are conducted by the author of this thesis. No part of this research work has previously been presented for any degree, either at this University or other institution known to me.

CONTENTS

## List of tables

## List of Figures

**AN INVESTIGATION OF THE ANOMALOUS LOW INCIDENCE OF MOTOR SIDE EFFECTS OF THE NOVEL ANTIPSYCHOTIC DRUG – Y931.**

# CHAPTER 1: General Introduction

## 1.1. Schizophrenia : Nature of the Disease

Schizophrenia is a highly complex heterogeneous psychotic disorder of the brain that affects normal thinking patterns, the perception of reality, and ability to express or feel emotions. Thus this disorder affects virtually all-higher domains of mental functions. This diversity of symptoms makes it difficult to explain this disorder in terms of any single type of dysfunction of any area of brain.

One person in a hundred develops schizophrenia in their late teens or early twenties, although it sometimes starts in middle age or even later in life. The characteristic time–course of the disease includes extensive illness followed by chronic disability. The degree of disability ranges from mildly diminished ability to cope with stress, to severe difficulties in initiating and organizing voluntary activity. Although equal numbers of males and females are affected, some data suggests, that males may have more severe manifestations of the disorder, including an earlier age of onset (by 2-4 years) with more marked neuropathological abnormalities, poor response to treatment, and less favourable outcome (Szymanski et al., 1995, Hafuer. et al., 1998).

The symptoms of schizophrenia are categorized and classified by the International Classification of Disease (ICD-10) and the Diagnostic and Statistical Manual of the American Psychiatric Association, $4^{th}$ edition (DSM-IV). The positive symptoms are those that reflect the mental process that is not present in normal individuals and include hallucinations and delusions, formal thought disorder, inappropriate affect, together with poor ability of social interactions because of reality distortion. Where as poverty of speech, loss of emotional responsiveness, reduction of motor function and social withdrawal are often referred to as negative symptoms as they represent deficits from normal function. Negative symptoms also include the loss of motivation, anhedonia and emotional vibrancy. Disturbances in cognitive functions such as attention, precise memory, organization of social life are also continually hampered. These can greatly

limit the patient's ability to function normally; lowering the quality of personal and social life causing concomitant mood symptoms such as depression and anxiety.

The criteria for diagnosis of schizophrenia can only be made, if the clinical features are present through out a period of one month and in addition, there would be marked impairment of social or occupational functioning and continuous signs of disturbed mental functions for at least six months.

| **Characteristic symptoms** | **Affective symptoms** |
|---|---|
| Reality distortion | Depression |
| Delusions | Low mood |
| Hallucinations | Low self-esteem |
| | Hopelessness |
| | Suicidality |
| **Disorganization** | **Psychomotor Excitation** |
| Formal thought disorder | Irritability |
| Disorganized or bizarre behavior | Pressure of speech |
| | Motor agitation |
| | |
| **Psychomotor Poverty** | |
| Flat affect | |
| Poverty of speech | |
| Decreased motivation | |
| Decreased voluntary motor activity | |
| | **Non-specific symptoms** – Anxiety |

Table 1. The symptoms of schizophrenia.

Although there was little or no evidence of a distinct relationship between symptoms and pathophysiology, the heterogeneity of the symptoms prompted many scientists to subtype the disease. Thus:

1) Paranoid schizophrenia characterized by reality distortion symptoms with relatively good preservation of personality.
2) Hebephrenia, which usually presents in adolescence or early adult life and is characterized by silly behaviour, fatuous affect, formal thought disorder and fragmented delusions.
3) Catatonia, which is dominated by episodic disorders of voluntary motor activity.
4) Simple schizophrenia characterized by gradual deterioration in function over a period of more than a year, without florid symptoms.

### 1.1.1 Time Course of Schizophrenia

The clinical course of schizophrenia suggests that the disease unfolds in distinct phases. Initially, there is a developmental insult to the CNS that does not result in psychosis. Evidence from the records of the children who developed the disease in later life, showed that most of them failed to learn the use of language within the normal time scale. Most of them also will be further behind in their schoolwork than their classmates. They are usually introverted as if they are shy. Thus they find it difficult to adjust with other children or adults around them (Done et al., 1994). These kinds of observations demonstrate an early association with subtle cognitive, motor function and social impairments, which may proceed to schizophrenia in the future.

## 1. 1. 2. Factors Influencing Induction of the disease.

Environmental factors such as, exposure to infectious agents, toxins and traumatic insults and stress during gestation and parturition (Dalman et al., 2001; Thomas et al., 2001) or childhood may play a role in the pathogenesis of schizophrenia.

Life events, especially during the growing up phase of teenage to adulthood, insult of harmful drugs or chemicals may cause specific CNS injury.

Family, twin and adoption studies have demonstrated that, the morbid risk of schizophrenia in relatives, correlate with the degree of shared genes. Regions of a number of chromosomes (Scordo et al., 2000 and Jones et al., 1994) have been implicated as sites of potential vulnerability (Lewis and Lieberman 2000; Pulver, 2000; Marcelis, et al., 1998, Brustowicz et al., 2000, Readler. et al., 2000). Obstetric complications (Dalman et al., 2001; Thomas et al., 2001) and various indicators of delayed development such as parental drug misuse during pregnancy (Heyser et al., 1994; Lipton et al., 1999; Stanwood et al., 2001), during infancy and childhood, may point to a developmental deficit in neuronal function. Whether or not these risk factors act on their own or in conjunction with genetic and environmental factors, remains to be fully established.

There is substantial evidence for a developmental etiology of schizophrenia (Johnstone et al., 1976; van Horn and McManus, 1992; Lawrie and Abukmeil, 1998; Marcelis et al., 1998; Lewis et al., 1997 & 2000; Jarskog et al., 2000; Raedler et al., 2000). Autopsy studies showed cellular abnormalities in the prefrontal and cingulate cortices of schizophrenic patients; a reduction in the number of GABA-containing interneurons; a reduction in the amount of GABA made by these neurons (but no loss in their number); a reduction in the dendritic processes of pyramidal neurons and a reduction in the terminal markers. In recent years, the development of brain imaging and positron emission tomography has allowed the brains of schizophrenic patients to be studied directly within a cellular and molecular context.

Thus, the cause of schizophrenia is likely to consist of genes conferring vulnerability and environmental insults, which express in the developmental maturation of brain circuitry. But the difference between schizophrenia and some of the other genetic neurodevelopmental diseases are that, there are no immediate overt manifestations of the disease. Rather most individuals appear to function normally until they enter the greatest period of risk in late adolescence and early adulthood. Thus, schizophrenia seems to be a polygenic disorder, associated with genetic environmental and developmental vulnerability factors.

From the discussion above it could be concluded that the interaction of an inherited predisposition to the disease condition and early neurodevelopment changes results in defective connectivity between some of the brain regions, e.g., midbrain, nucleus accumbens, subthalamic nucleus, thalamus, temporolimbic and prefrontal cortex (Jones, 1997). This defective neural circuitry is then cause abnormal function expressing the disease-state of the individual.

## 1.1.3. Treatment of Schizophrenia

Schizophrenia was first treated with neuroleptic agents after the discovery of chlorpromazine in 1952 (Delay and Deniker, 1952). A number of studies compared the effects of these first generation antipsychotic drugs with those of general sedatives such as barbiturates that were in use before. These studies had shown that these compounds have a specific antipsychotic action that is not possessed by general sedatives (Klein and Davis, 1969). They have a completely different action to which the fundamental symptoms of schizophreniawhich are uniquely characteristic of the disorder, responded selectively. This dramatically improved the treatment of schizophrenic patients. These medications calmed down the agitated patients and greatly modified the positive symptoms of schizophrenia effectively.

These drugs (Clorpromazine and Phenothiazine) were developed, using motor side effects such as catalepsy as an end point in the laboratory animals, because such effect were believed to be closely related to the therapeutic mechanism of the components of the drugs. Similarly, clinicians commonly used movement disorders in the patients, as an indicator of antipsychotic effects (Glazer, 2000). Thus the defining criteria of neuroleptics was the induction of an extrapyramidal syndrome. The drug that was discovered around this time was from Rauwolfia serpentia [(locally known as *shawrpa gondha*) used as medication for insane peoples for its tranquilizing properties] first described and used by Sen and Bose 1931. This was possible after isolation of the physiologically active ingredients responsible for treatment of psychiatric disorders. The new drug was marketed as reserpine. The similarities between the effects of reserpine on mental patients and that of phenothiazine were evident to Deniker and Delay. This drug also caused motor side effects at therapeutic doses identical to that of chlorpromazine, despite a very different molecular structure. This gave an incentive to find other compounds that could be similarly useful. In 1959 haloperidol, a non-selective dopamine antagonist, was introduced to the market. This was the neuroleptic to be used from then on for hallucinations, paranoia, delusions, etc., as in schizophrenia (Janssen, 1967, 1970;

Lewi P J et al., 1970). All these antipsychotic drugs are dopamine $D_2$ receptor antagonists. The mechanism of action of these neuroleptic drugs used in the treatment of schizophrenia was generally considered as the blockade or disruption of cerebral dopaminergic mechanism, in 'mesolimbic dopamine' (Costall and Naylor, 1975; Millar et al., 1974; Carlsson et.al., 1978). They possess clinical efficacy in the treatment of positive symptoms and their ability to inhibit amphetamine-induced stereotypy (Garver et. al., 1975). And these drugs are categorised as typical antipsychotic drugs.

However, the limitations of these agents became clearer within a few years. Neuroleptic agents inhibit other dopamine systems and, of these, the antagonism of striatal dopamine mechanisms was considered to play an important role in the development of Parkinsonian-like syndromes (Hornykiewicz, 1973). And this accused the major disadvantage of these neuroleptic drugs such as the development of a variety of movement disorders termed extrapyramidal side effects (EPS). More than 80% patients treated with the first generation neuroleptics expected to develop significant EPS that will often lead to non- compliance with medication. Although there was a gradual decline in the number of hospitalised patients, it took a few years to realise the effect of the extrapyramidal syndrome on the patients. Most patients released from hospital were not considered 'cured', but were in some stage of remission. Thirty percent of patients may had been considered to be completely recovered, 60% may have experienced varying degrees of recovery, while 10% of schizophrenic patients failed to respond to treatment. And relapse rate was high. The blockade of mesolimbic $D_2$ receptors was believed to relieve positive symptoms (Carlsson et.al., 1978) but the difficulty was, treatment with typical antipsychotic drugs generated EPS.

All these reasons encouraged researchers to find other compounds that could have greater efficacy on the negative symptoms of schizophrenia and to cause fewer or none of the unwanted extrapyramidal side effects. The research resulted in the discovery of clozapine, which was patented in 1960. Clozapine became the model for newer drugs with the characteristics: 1) great efficacy on the negative symptoms of schizophrenia with

2) very few, if any at all, extrapyramidal side effects (Van Praag, et. al., 1976; Baldessarini and Frankenberg, 1991).

Although all the drugs, which are used to treat psychosis, are now termed as 'antipsychotic drugs', the earlier drugs are also called 'typical' antipsychotic drugs as these are also associated with extrapyramidal side effects. Drugs that are developed on the basis of clozapine as a model for their characteristics are termed as 'atypical antipsychotic drugs' with fewer and less pronounced occurrence of EPS.

Subsequent research on the properties of chlorpromazine and haloperidol suggested that these compounds' antipsychotic actions depend on their capacity to block $D_2$ dopamine receptors. According to the 'dopamine hypothesis', schizophrenia is caused by excessive dopaminergic neurotransmission or high level of $D_2$ – like receptors in the brain. Thus the ability to block postsynaptic $D_2$ receptors is the characteristic biochemical effect of all classical antipsychotics.

Thus it was proposed that neuroleptics like chlorpromazine and haloperidol block the post-synaptic $D_2$ receptors. Seeman et al. (1976) linked the efficacy of these drugs to their $D_2$ receptor binding affinity. And in recent years (Kapur et.al.,2000b; Farde et.al., 1992), positron emission tomography (PET) confirmed the correlation between the $D_2$ receptor occupancy rate and the clinical efficacy and onset of EPS.

Few remedies have been found for these neuroleptic-induced movement disorders, which had prompted research to look for drugs with better efficacy.

Clozapine is the prototype atypical antipsychotic drug that produces no EPS or very few (Costall and Naylor, 1975) if any at all and appears to be effective in reducing many symptoms of schizophrenia (Meltzer and McGurk, 1999; Lieberman et.al., 1994; Lee et. al., 1994). These characteristics of clozapine led many authors to seriously question the inseparability of antipsychotic-Parkinsonian like effects (Costall and Naylor, 1975). Thus it was believed that in the core of atypicality is a reduced liability of EPS and less or

devoid of other adverse effects of the drugs, such as – sedation, autonomic movements, sexual arousal and other adverse effects. Therefore a clozapine like drug (an atypical antipsychotic) without its harmful haematological side effects will be most helpful to the patients with schizophrenia. Clozapine has high affinity for $D_2$ dopamine receptors but,

| | Atypical ADP | Typical ADP |
|---|---|---|
| 1. | Clozapine | Amisulpride / Sulpiride |
| 2. | Olanzapine | Clorpromazine |
| 3. | Risperidone | Reserpine |
| 4. | Perspirone | Haloperidol |
| 5. | Sertindole | Loxapine |
| 6. | Ziprasidone | Thioridazine |
| 7. | Zotepine | Proclorperazine |
| 8. | Tiospirone | Perphenazine |
| 9. | Fluperlapine | Mesoridazine |
| 10. | Tenilapine | Triflurperidol |
| 11. | Quetiapine | Thiothixene |

Table 2. List of some of the most-widely prescribed antipsychotic drugs in the market.

not as high as the typical APD. It also has very high affinity for various type of 5-hydroxytryptamine (5-HT) receptors, most notably the 5-$HT_2$ receptors. In recent years more receptors for dopamine and serotonin receptor subtypes have been discovered e.g., $D_3$, $D_4$; 5-$HT_{2A}$, 5-$HT_{2B}$, 5-HT $_{2C}$ , 5-$HT_6$ , 5-$HT_7$. This has made the receptor pharmacology of clozapine very complex. Other than serotonin, clozapine also has high affinity for adrenergic, muscarinic, and histaminergic receptors (Meltzer and McGurk, 1999).

One view of the mode of action of atypical antipsychotic is that, these drugs interact with brain 5-$HT_{2A}$, or 5-$HT_{2B}$ or 5-$HT_{2C}$ serotonin receptors, while also occupying dopamine $D_2$ receptors. One of the characteristics of these drugs is that they bind to 5-$HT_2$ with a higher affinity than to $D_2$ receptors. It is thought that there is a definite ratio between 5-$HT_{2A}$ and $D_2$ receptor affinity for their atypical anti psychotic properties (Meltzer et.al,

1989,1991; Kapur et. al.1999, 2000a; Wadenberg et. al., 1997; Neal-Beliveau et.al., 1993). These drugs are also referred as serotonin, dopamine inhibitors (SDI).

The quest for finding new drugs with similar pharmacology as for clozapine and new knowledge from the study of clozapine helped to produce a number of new generation antipsychotic drugs, such as: risperidone, olanzapine, quetiapine, ziperasidone, seritindole and so on in the recent years. Like clozapine, therapeutic doses of quetiapine cause very few or minimal EPS (Casey, 1996, 1997).

| DRUGS | Receptor affinity (Ki, nmol/L) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D1 | D2 | D3 | D4 | 5-HT 1A | 5-HT 2A | 5-HT 2C | α -1 | α-2 | M-1 | M-3 | M-4 | $H_1$ |
| Cloz | 190 | 220 | 92 | 43 | 120 | 5.0 | 4.8 | 3.6 | 300 | 47 | 30 | - | 9.8 |
| Olanz | 45 | 28 | 51 | 38 | 1600 | 2.6 | 1.2 | 11 | 1200 | 57 | 210 | 120 | 24 |
| Y931 | 36 | 2.5 -4.5 | 1.9 | 20 | 230-630 | 1.7-9.62 | 9.62 | 1.5-3.1 | 340 | 12 | 7.1 | 20 | 36-54 |
| Halop | 150 | 2.0 | 2.7 | 3.7 | 1200 | 59 | 5800 | 5.5 | >1000 | >1000 | - | - | 3900 |

Table 3. Comparison of binding affinities of Y931 with Clozapine, Olanzapine and haloperidol for various receptors that may be involved in extrapyramidal side effects (EPS) [Meltzer et al., 1989; Arnt and Slearsfeldt., 1998; Hilger and Kasper., 2000]

It is believed that atypical antipsychotic drugs preferentially activate the dopaminergic systems of limbic structures but not the striatum (Gessa et.al., 2000; Jaskiw et. al., 2001; Chen et.al., 1991).

Y931{8-fluoro-12-(4-methylpiperazin-1-yl)-6H[1]benzothieno[2,3b][1,5]benzodiazepine maleate} is a new multi-affinity compound with a similar pharmacology to clozapine (Morimoto et al, 2002).. However, despite its higher affinity for D2 receptors than clozapine and other antipsychotic drugs, it appears to exhibits few EPS in preliminary studies (Morimoto et al, 2002).

## 1.1.4. Extrapyramidal syndrome: A side effect of treatment with antipsychotic drugs

Since 1950s the patients diagnosed with schizophrenia, are treated with antipsychotic drugs, mostly haloperidol. Although it reduces overall severity of the illness, using the drug for longer periods, (within the "optimal dosage") brings on the motor side effects, along with the other side effects. The motor side effects caused by neuroleptics are categorised as extrapyramidal side effects because they relate to the drug actions outside the pyramidal motor system (Schillevoort, et al. 2000) such as the striatum, globus pallidus and substantia nigra, which control voluntary movements. PET analysis of patients with acute EPS indicates that it is caused due to occupancy of at least 80% of $D_2$ receptors in the basal ganglia (Farde et.al., 1992). EPS normally occurs in acute and late onset (tardive) forms. Most of these symptoms undergo remission after the drug treatment stops.

In normal condition there is a balance between dopaminergic and cholinergic tone in the striatum. In schizophrenia, this balance is disturbed due to dopaminergic hyperactivity.
It is now generally accepted that the ability of the 'typical' or early generation anti psychotic drugs (APD) and also the newer 'atypical' antipsychotic drugs (to a lesser extent), to cause extrapyramidal side effects can be correlated with their high affinity to the $D_2$ dopamine receptors in the striatum. Blockage of the $D_2$ dopamine receptors by the typical antipsychotic drugs, increases the activity of the indirect (excitatory) striatal output pathway to the substantia nigra via the globus pallidus, and subthalamic nucleus and decreases the activity of the direct (inhibitory) pathway to the substantia nigra. These results in increased descending output from the substantia nigra, causing disturbances in the motor circuits, effecting Parkinson's like side effects known as the extrapyramidal side effects. Knockout mice lacking $D_2$ receptors, but not those lacking $D_1$, $D_3$ or $D_4$ receptors, exhibit catalepsy, the experimental counterpart of Parkinsonism. Thus antagonism of the $D_2$ and $D_3$ receptor ($D_2$ like receptors) but not the other dopamine receptors, is the most likely cause of neuroleptic-induced Parkinson like syndrome.

Extrapyramidal side effects can take a variety of forms. There are four major categories of EPS.

a) Parkinsonism
b) Tardive dyskinesia (TD)
c) Dystonia
d) akathisia

a) Parkinsonism consists of a parkinsonian syndrome that resembles true Parkinson disease. The symptoms include tremors in hand and arms, rigidity, spasticity or difficulty in initiating movements, and akathesia or restlessness, emotions are less expressive. Patients could experience this symptom within a very short time, within days or weeks after the treatment starts.

b) Tardive dyskinesia (TD) is a more severe disorder of movement of muscles. It affects face and neck irregular involuntary muscle movements, usually in the face around the mouth. But it could also be in the arms, legs and body. TD develops after prolonged exposure to the neuroleptics and can become irreversible. TD is the most severe side effect of the first generation neuroleptics and believed to be caused by super sensitivity response to chronic dopamine blockade. It is very common after repeated drug treatment and is not readily reversible after cessation of drug treatment. Because of this reason, TD has severely limited the use of classical neuroleptics.

c) Dystonia is a sudden and severe spastic movement of muscles of face and neck. Acute dystonia can occur during the second or third day of treatment.

d) Akathisia is a syndrome charecterised by a subjective sense of anxiety and restlessness. EPS-related akathesia is often mistaken for psychotic agitation.

When all the parameters are fully explored, it was found that each form of EPS has unique characteristics.

Y-931, 8-fluoro-12-(4-methylpiperzin-1-yl)-6H[1]benzothieno[2,3b][1,5]benzodiazepine maleate, is a very new multi-affinity compound, with similar receptor profile like clozapine, olanzapine and resperidone. Y931 also interacts with multiple neurotransmitter receptors such as dopaminergic, serotonergic, α-adrenergic, muscarinic and histaminergic receptors. Y931 is active in a dose-dependent manner in inhibition of apomorphine-avoidance responses. It has the property to ameliorate NMDA receptor hypofunction. It has potent protective action against the dizocilpine-induced neurotoxicity in the retrosplenial cortex and it has power to reverse dizocilpine-induced social deficits at the same doses at which it is neuroprotective (Morimoto et al, 2002).

The preliminary experiments seem to indicate that Y931 has the important property of an atypical antipsychotic drug with very few EPS, despite its higher affinity for $D_2$ receptors than clozapine and some other atypical antipsychotic drugs. Thus finding the exact site / sites of the physiological action that attenuate the effect causing EPS, could be of major significance for the understanding of Y931's pharmacology, and for the development of antipsychotic drugs with improved efficacy.

The aim of this project is to try and uncover this aspect of the characteristics of the Y-931 compound.

## 1.1.5. Brain regions involved in extra pyramidal syndrome:

Brain regions that are commonly implicated with schizophrenia are: 1) Temporal and parietal association cortices, 2) Prefrontal cortex, 3) Anterior cingulate cortex, 4) Basal ganglia, 5) Thalamus, and 6) Ventral mesencephalon (Lewis and Lieberman, 2000).

Motor activity is controlled by three major systems: cerebral cortex, basal ganglia and cerebellum. The basal ganglia are a group of subcortical nuclei of the vertebrate brain that are intimately involved in the control of movement. The organisation and connections of rat basal ganglia had been shown to be very similar to those in the human basal ganglia.

Drug-induced EPS thought to be mediated at the level of the basal ganglia. The diagram on page 31 and 46 illustrates the different brain structures of the basal ganglia and how these relate to the other brain regions.

The grey matter that lies deep within the cerebral hemisphere forms the subcortical nuclei or basal ganglia, which are the important structures involved in the control of extra-pyramidal movement. These include striatum (caudate/putamen and ventral striatum), globus pallidus (internal-GPi, and external-Gpe), subthalamic nucleus (STN) and substantia nigra [a) pars compacta (SNc) and b) pars reticulata (SNr). They are a complex and highly interconnected group of nuclei. In the rat GP is a single entity equivalent to the GPe in the primates and connected to the entopeduncular nucleus (EP) (Parent and Hazrati, 1995; Smith et al., 1998; Heimer et al., 1995).

The caudate/putamen have identical cell types as they are both developed from the same telencephalic structure and are anteriorly joined together. These two structures together are known as neostriatum or striatum. They are the input nuclei (or gateway) for the basal ganglia and receive fibers from all functional parts of cerebral cortex and intralaminar

ganglia and receive fibers from all functional parts of cerebral cortex and intralaminar nuclei of the thalamus. The cortico-striatal projection originates from neurons located in both supra-granular and infra-granular cortical layers in each cortical

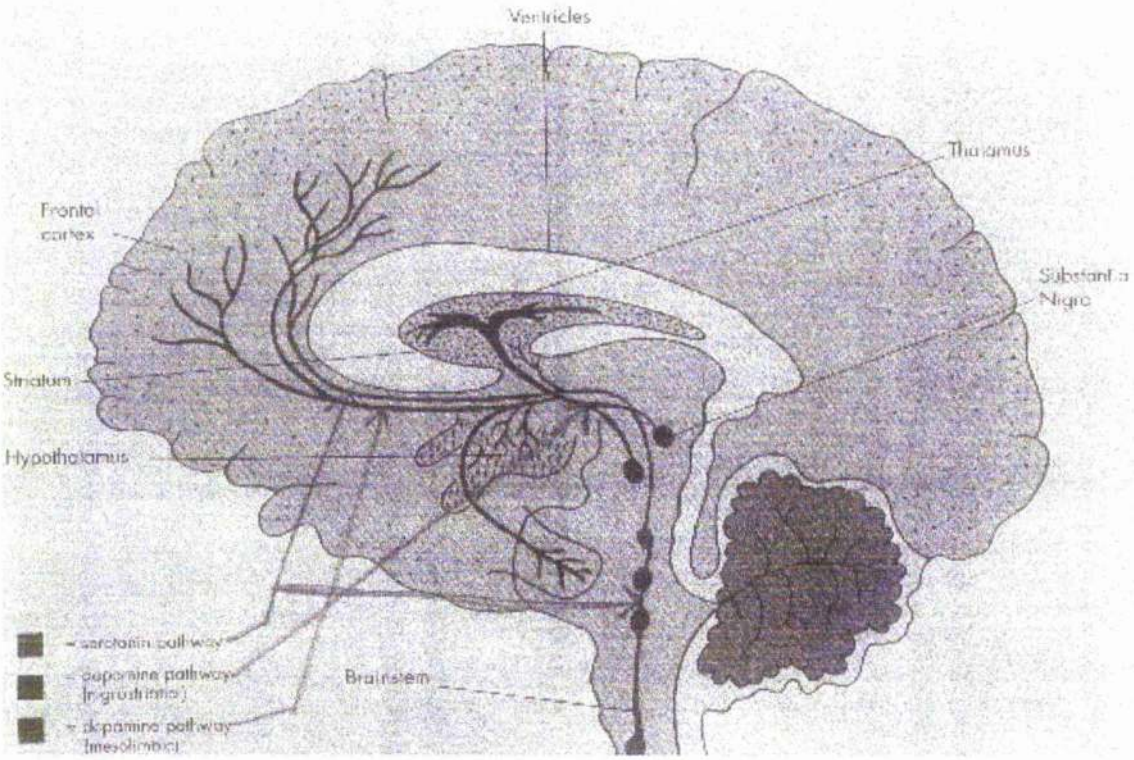


Fig.1. Functional anatomy of brain regions involved in the dopamine and serotonin pathways (Adopted from 'Target Schizophrenia' – The association of the British Pharmaceutical Industry Edited by B. Kirkness, 1997).

region. The cortico-striatal projection originates from neurons located in both supra-granular and infra-granular cortical layers in each cortical region. The decrease in the number of neurons in the striatum compared to the cortex suggests a high degree of convergence in the organisation of the cortico-striatal projection. A feature of the cortico-

striatal system is the marked heterogeneity of its terminal fields in the form of clusters of various sizes - the striosomes (or patches) and the extrastriosomal matrix. Striosomes receive afferents mostly from prefrontal and limbic cortices. The neurons projecting from the cortex are glutamatergic.

## Striatum

The striatum is composed of both projection neurons (Golgi type I cells) and local interneurons (Golgi type II cells). Projection neurons have a medium-sized cell body with 4-5 dendrites, which contain numerous spines (hence medium spiny neurons) and they are GABAergic and also coexpress substance P, enkephalin, dynorphin and neurotensin. The medium spiny neuron is the main integrating element of the striatum, thus they are the major target of both local and extrinsic afferents. The medium spiny neuron integrates information that comes from a wide variety of afferents and conveys the result of this complex neuronal computation outside the striatum. Interneurons on the other hand have none or rarely any spines on their dendrites. A small number of the interneurons are GABAergic and also contain parvalbumin. The majority of the interneurons contain somatostatin, neuropeptide Y, (NADPH)-diaphorase, which is involved in the synthesis of nitric oxide (NO) and use acetylecholine as neurotransmitter. But they do not express GAD mRNA.

The striatum receives an innervation from the substantia nigra pars compacta and dorsal raphe nucleus. They are dopaminergic and serotonergic respectively. Efferent fibers from the striatum terminate mainly in the substantia nigra reticulata and in the lateral and the medial segments of the globus pallidus and entopeduncular nucleus (GPi) and also the subthalamic nucleus in primates and human.

The efferents from the ventral striatum (accumben core and shell) project to the ventral and ventromedial pallidum, entopeduncular nucleus and lateral part of the ventral tegmental area. This suggests that the ventral striatum can affect the activities of the dorsal striatum via the mesencephalic dopamine system.

## Globus pallidus

The globus pallidus or pallidum is derived from diencephalon. In the human, it is divided into internal (GPi) and external segments (GPe). There are at least two types of neurons in the globus pallidus, spiny and aspiny, displaying different dendritic fields. The dorsolateral part of globus pallidus is richer with cytochrome oxidase, and ventromedial part is rich with acetyl cholin-esterase.

The globus pallidus-internal (GPi) and substantia nigra reticulata constitute the major output nuclei of the basal ganglia. Both structures project to specific thalamic relay nuclei, the ventral anterior and ventral lateral nuclei, which in turn project back to the cerebral cortex.

One of the most prominent efferent projections from globus pallidus is to the subthalamic nucleus. The other important targets of the GPi efferent pathways include projections to the mediodorsal and reticular thalamic nucleus and the lateral hebanula at diencephalic levels, as well as the pedunculopontine tegmental nucleus at mesopontine brainstem levels and substantia nigra (both SNRand SNCD). In rat, the important targets of the globus pallidus projections include superior colliculus. In rat individual pallidal neurons may send collateral branches to the striatum, and the substantia nigra pars reticulata (Staines and Fibiger, 1984).

The ascending projections of pedunculopontine tegmental nucleus provide a massive innervation to the substantia nigra compacta, subthalamic nucleus and pallidum.

Thus the major outflow of the basal ganglia in rat is directed through the entopeduncular nucleus (GPi) and substantia nigra reticulata neurons, which send many collateral axon branches to the thalamus and brain stem (Parent et al., 1983; Bentivoglio et al., 1979). These two structures share many histological characteristics. In the rat, the entopeduncular nucleus and nigral projections overlap (Smith et al., 1998).

## Substantia nigra

The substantia nigra lies in the midbrain and has two zones – a) ventral pale zone, the pars reticulata, b) dorsal darkly pigmented zone, the pars compacta. The lateral margin of the rostral half of the SNR exclusively contains the nigro-tectal cells and is called substantia nigra pars lateralis (SNL). The internal segment of globus pallidus and substantia nigra pars reticulata have very similar cell types, connectivity and function, they are sometimes regarded as a single structure. Various projection neurons in the substantia nigra pars reticulata receive input directly from the striatum (Williams and Faull, 1985) and from entopeduncular nucleus or GPi (Smith et al., 1998: Heimer et al., 1995).

The substantia nigra gives rise to the descending projections to the pedunculopontine tegmental nucleus, as well as to the superior colliculus.

## Subthalamic nucleus

The subthalamic nucleus (STN) lies below the thalamus at its junction to midbrain, dorsal to the cerebral peduncle between substantia nigra and entopeduncular nucleus (EP) in the rat. Neurons in the subthalamic nucleus are immunopositive to glutamate rather than GABA ergic, as previously thought (Robledo and Féger, 1990). This nucleus exerts an inhibitory effect on the output nucleus of basal ganglia, and absence of this control due to any lesion was thought to be the cause of ballism, characterised by involuntary movements (Smith et al., 1998).

Bevan et al., (1994) demonstrated that neurons of the striatum (direct pathway) innervate common regions of the EP/Gpi or SNR. Terminals from striatum and STN (indirect

pathway) form convergent synaptic contacts with individual dendrites and perikarya in both EP and SNR. Some of these neurons project to the ventral medial nucleus of the thalamus (Bevan et al., 1994). The same authors had shown that individual output neurons in the EP or SNrRthat receive synaptic input from striatum also receive synaptic input from terminals derived from the STN and GP. Thus groups of neurons in the Gpi / EP, STN and SN are likely to be reciprocally connected. (van der Kooy et al., 1980). The intralaminar nuclei of the thalamus, the dorsal raphe, the mesopontine tegmentum and the dopaminergic cells in the SNCD also innervate the STN (Bevan et al., 1995).

Although the striatum is commonly seen as the main entrance of cortical information to the circuitry of the basal ganglia, the STN also receives excitatory glutamatergic projections from the cerebral cortex (Bevan et al., 1995; Kitai and Deniau, 1981). Thus STN have the morphological and neurochemical characteristics of pallidal terminals (Bevan et al., 1995). This corticosubthalamic projection is exclusively ipsilateral and largely derived from the primary motor cortex with a minor contribution from prefrontal and premotor cortices. These projections are topographically organised. Corticosubthalamic neurons are mainly located in the layer V and many of them send axon collaterals to the striatum (Smith et al., 1998).

Thus the main input to the basal ganglia comes from frontal cortex and its output returns, via the thalamus, to the frontal cortex. The new information (program) is then transmitted via descending pathways to the lower level of motor control system in the brain stem and /or spinal cord course. Most motor programs are constantly adjusted during each manoeuvre to make the process perfect according to circumstances.

There are two connections (pathways) from the basal ganglia: 1) direct and 2) indirect, to the motor cortex /sensory motor cortex. The direct pathway produces the desired output activity by increasing and maintaining the initial activity and inhibiting unwanted movement through its influence via thalamus. It contains $D_1$ dopamine receptors, which control the direct pathway by reducing the inhibitory actions. The indirect pathway is

responsible for sorting out unwanted motor activity by decreasing or submerging it. It contains dopamine $D_2$ receptors, which control the hyper-activity of striatopallidal neurons and subsequent reduction of the tonic inhibitory influence of the globus pallidus (GP) on the subthalamic nucleus (STN). The substantia nigra modulates the activity of the striatum. It activates the direct pathway, and inhibits the indirect pathway. But a damaged substantia nigra cannot maintain this balance. Lost or damaged neurons in the substantia nigra will cause loss of dopaminergic innervation, causing dominance of the indirect pathway, thus resulting in Parkinsonism or Parkinson like symptoms known as EPS.

## 1.1.6. Neuro-transmitters and neuropeptides involved in EPS.

Neurotransmitter release is highly $Ca^{2+}$ -dependent. Voltage sensitive $Ca^{2+}$ channels are essential to transmitter release into the synapse. Membrane fusion that results in neurotransmitter secretion is the basis of neural communication.

A diagram illustrating the location of different neuro-transmitters and neuropeptides in the basal ganglia is shown in Fig.2 (page-46). Details of the various neuro-transmitters and neuropeptides are summarised in the following sections.

### Excitatory and inhibitory Amino Acids

The major excitatory neurotransmitter in the brain is glutamate (Glu). The major inhibitory neurotransmitter is γ-aminobutyric acid (GABA). The corticostriatal pathway is excitatory via the amino acid transmitters, glutamate (Glu) and aspartate. GABA hyperpolarises cells rapidly, whereas Glu depolarises neurons rapidly. These amino acids mediate most of the fast synaptic transmission in the brain. These two amino acid neurotransmitter pools are not generally colocalised. However, in some brain regions, isolated neurons are seen in which GABA and Glu have reported. The excitatory neurotransmitter glutamate not only mediates point to point transmission, but also carries a more diffuse signal in the perisynaptic environment. Synaptically released glutamate can activate pre- or postsynaptic metabotropic receptors, many of which are located outside the synaptic cleft. Glutamate can also reach kainate or metabotropic receptors located on the GABA- releasing axons of interneurons.

GABA is present in highly diverse inhibitory neurons and projection neurons throughout the brain. Cortico-striatal fibres terminate on medium spiny neurons, the output cells of the striatum. All the medium spiny neurons are inhibitory and release GABA.

Approximately 95% of striatal neurons are thought to be GABA-ergic projection neurons. GABA co-exists with neuropeptides in various combinations in principal circuits and in interneurons of the basal ganglia. The striatum has two different kinds of projections: a) one class projects directly to the output nuclei of basal ganglia; b) the other class projects indirectly to the output nuclei through external globus pallidus and subthalamic nucleus. GABA has been implicated in the regulation of dopamine-mediated events in the extrapyramidal system (Ossowska et al., 1984).

The synthesis of GABA requires a critical biosynthetic enzyme glutamic acid decarboxylase (GAD). GAD is the rate-limiting enzyme in the synthesis of GABA and catalyses the decarboxylation of glutamic acid to produce GABA. This enzyme is found exclusively in neurons in which GABA is neurotransmitter. GAD is not present in glia.

.Molecular cloning studies have shown that there are two isoforms of GAD - designated GAD65 and GAD67 according to their molecular weights, exhibit somewhat different intracellular distributions and are encoded by two genes, suggesting that the two isoforms may be regulated in different ways. These isoenzymes exhibit over 90% aminoacid sequence homology across the two species. GAD65 is more common in nerve endings and is particularly enriched in nerve terminals and thus may be somewhat more important for GABAergic transmission (Erlander et al., 1991). Morever, GAD67 is found in the cytoplasm. Increased demand for GAD67 usually involves the synthesis of new enzyme, as most of GAD 67 enzyme might be active and able to synthesize constant levels of GABA in GABA containing neurons (Soghomonian and Martin 1998). Glutamic acid is normally returned to neurons, where it regenerates GABA in the presence of GAD. Thus the presence of GAD67 is a marker for GABAergic neurons. Glutamatergic neurons do not use GABA as a transmitter (Erlander et al., 1991; Martin et al., 1993).

Both isoforms of GAD are expressed in GPi (entopeduncular nucleus, EN in rodent), SNR and GPe. GAD 67 mRNA expression changes in Parkinson's disorder and also in 6-OHDA lesioned rats.

Thus GAD67 is an indirect marker of GABAergic activity within the basal ganglia. GAD67 mRNA expression has been taken as an index of the GABAergic activity in GPe neurons. Since GAD67 levels reflect long-term changes in GABA neurotransmission it could be used to determine the steady-state level of GABAergic activity in GPe neurons in semi-chronic or chronic diseases of the basal ganglia (Levy et al., 1997).

## Catecholamines

In the brain, only 0.0005% of the cells use catecholamines as neurotransmitters. These belong to a wider group of neuro-transmitters, called monoamines. They are derived from amino acids. Catecholamines are metabolised by monoamine oxidase (MAO), which exists in two forms, MAO-A and MAO-B and by catechol-O-methyl transferase. Because most of the normal brain functions are so much dependent on these neurotransmitters and the neurons that contain them, enormous research efforts has been devoted on them as compared to other neurotransmitters.

The most prominent of catecholamine molecules are dopamine, noradrenaline and adrenaline neurotransmitters. Dopamine and noradrenaline are derived from tyrosine. Tyrosine hydroylase is the rate-limiting enzyme in catecholamine biosynthesis. The catecholamine biosynthesis is regulated by phosphorylation of at least four distinct serine residues by at least nine distinct protein kinases. Actions of synaptic catecholamine are terminated by specific transporter: the noradrenaline transporter and dopamine transporter. The uptake of catecholamines into synaptic vesicles for release is accomplished by distinct vesicular monoamine transporter proteins.

Although monoamine containing CNS neurons are concentrated in the brainstem, monoamine neurotransmitters exert modulatory effects on almost all circuits in the brain, acting to dampen or facilitate communication among neurons and to regulate the plasticity of these circuits.

Dopamine neurons have cell bodies in the midbrain within the substantia nigra pars compacta and the ventral tegmental area and in the hypothalamus. Dopamine (DA) is also

produced by local circuit neurons in the retina. Both DA and L-DOPA (the precursor of DA) may cause changes in the metabolism and storage of serotonin in the brain. It is known that L-DOPA may accumulate in the serotonin nerve terminals and decreases the turnover of serotonin. On the other hand DA in the caudate nucleus can be reduced by large doses of 5-hydroytrytryptophan (5-HTP), the precursor of serotonin (5-HT). Thus DA may be displaced, or released from DA neurones by newly formed 5HT (Kostowski et al., 1972). Midbrain dopamine neurons projecting to the forebrain influence motor control, emotion, motivation and cognitive processes, including multiple forms of memory. Hypothalamic dopamine neurons regulate neuroendocrine function, most notably inhibiting the synthesis and release of prolactin.

There are only 12500 noradrenaline containing neurons in the locus coeruleus in the human brain, and even fewer than these in rodent's brain. But more and more, it is evident that they are very important for the overall function of brain. Noradrenaline is synthesised in a small number of brain stem nuclei, e.g., locus ceruleus in the dorsal pons. Noradrenaline is believed to play important roles in attention, learning, memory and many more physiological activities. These neurons have widely distributed axons that project to virtually every areas of the brain. Thus changes in the neuronal activity of these cells can alter noradrenaline release through vast areas of brain. Noradrenergic mechanisms have been implicated in the modulation of catalepsy, as noradrenaline hyperfunction antagonises the cataleptogenic effects of haloperidol (Toru et al., 1985) or EPS.

Adrenaline also occurs in a very small number of cells in CNS in the lateral tegmental region and in the dorsal medulla. These cells may be involved in the control of movement, although the functions of these cells are not well understood.

## Serotonin

Serotonin is the third major monoamine transmitter. It is also known as 5-hydroxytryptamine (5-HT). Serotonin is an indolamine that possesses a hydroxyl group at the 5 position and a terminal amine group on the carbon chain. It is synthesised from the amino acid tryptophan. Tryptophan is actively transported across the blood-brain barrier and hydroxylated by tryptophan hydroxylase producing 5-hydroxytryptophan, which is then decarboxylated by L-aromatic amino acid decarboxylase (enzyme, that is also involved in the biosynthesis of catecholamines) and the end product is serotonin. Tryptophan hydroxylase is a rate-limiting enzyme. This enzyme also belongs to the same family of amino acid hydroxylase as tyrosine hydroxylase for catecholamine. Both these enzymes can be activated by protein kinase A, cAMP and also by a calcium / calmodulin-dependent protein kinase. Serotonin reuptake is mediated by a serotonin transporter. There is only one gene for the human serotonin transporter, with 48% homology to both noradrenaline and dopamine transporters at the amino acid level.

Neurons that synthesise and release serotonin (5-HT) are found almost exclusively in the raphe nuclei of the brain stem. The dorsal raphe and median raphe innervate much of the rest of the CNS by means of numerous and sometimes diffuse projection pathways.

## Histamine

Histamine is produced in the posterior hypothalamus. Recent research indicates that it also serves important functions in the CNS. The neurons that produce histamine project to regions through the brain and spinal cord synaptically where it acts on at least three receptor subtypes. Areas that receive especially dense projections include the cerebral cortex, hypocampus, neostriatum nucleus accumbens, amygdala and hypothalamus. Many psychotropic medications including the antidepressants and antipsychotics (clozapine) cause sedation partly because they are potent histamine $H_1$ receptor antagonists. Histamine is produced by the decarboxylation of the amino acid histidine by

histidine decarboxylase, which is also involved in the synthesis of catecholamines and serotonin. Histamin has been implicated in the regulation of perception of pain, prevention of emotional sickness. However actions of this neurotransmitter in the nervous system not yet clearly understood.

## Acetylcholine

Acetylcholine was the first molecule to be identified as neurotransmitter. Acetylcholine is produced in the CNS by projection neurons, whose cell bodies are located in the basal brain stem and basal forebrain. Ach is also synthesised in interneurons in many brain regions including the striatum. The basal ganglia receive cholinergic input from the nucleus basalis, the pedunculopontine nucleus and laterodorsal tegmental nucleus. Innervation is intrinsic. Innervation in this region also arises from large cholinergic interneurons, which are critical components of the complex circuitry that underlies extrapyramidal motor control.

In normal condition, there is a balance between cholinergic and dopaminergic systems in the basal ganglia. And an enhancement of the cholinergic component might yield an inhibition of dopaminergic neurotransmission causing catalepsy in animals.

## Nitric oxide

Nitric oxide (NO) is an endogenously synthesized effector molecule which acts as a neurotransmitter with novel properties in the central and peripheral nervous systems. It is a free radical. Its involvement as a mediator of cell death in neurodegenerative diseases is well known. In the central nervous system, nitric oxide synthase (NOS) is expressed in

select neural populations. NOS distribution in the brain regions are detected in subthalamic nucleus, putamen, caudate nucleus, globus pallidus, claustrum and overlaying insular cortex. NOS positive neurons were not found in the substantia nigra and no signal was detected in the thalamic levels. In subthalamic nucleus, more than 95% of neurons express NOS mRNA. Whereas in the caudate and putamen only 1.5 – 2% of the total neurons express NOS mRNA. Because of its specific distribution, NOS mRNA expression can be used as a marker gene for the subthalamic nucleus and the striatum.

## Neuropeptides and calcium binding proteins

Neuropeptides are small proteins that serve neurotransmitter like functions in the nervous system. They serve primarily modulatory roles in the nervous system and usually interact with G- protein coupled receptors. Synthesis of neuropeptides requires the transcription of DNA and translation of the resulting mRNA into protein. The receptors for neuropeptides bind to their ligand with much greater affinities than do the receptors for small-molecule transmitters.

Neuropeptides are synthesised differently than neurotransmitters, as large precursor polypeptides that undergo extensive post-translational processing that includes cleavages into smaller peptides, the prepropeptide and subsequent enzymatic modification into an active peptide. Tachykinins, a family of neuropeptides' has several members derived from two preprotachykinin genes. Neurokinin A and B, and substance P are members of this family.

The medium spiny neurons contain several neuropeptides:

a) Tachykinin system: - Substance P and neurokinin A and B
b) Opioid system:-Dynorphin and Enkephalin

Expression of these substances is regulated by the dopamine input to the striatum. Substance P and dynorphin are the co-transmitters with GABA in medium spiny neurons

in the direct pathway from the striatum. These cells of the direct pathway contain dopamine $D_1$ receptors. Enkephalin is the co-transmitter with GABA in the medium spiny neuron derived indirect pathway to the external globus pallidus. These cells express dopamine $D_2$ receptors.

The distribution of calbindin D-28k (CB) and parvalbumin (PV), two calcium binding proteins, in the neurons and fibres in the mammalian central nervous system has been widely investigated (Baimbridge, et.al. 1992; Celio, et.al.1990; Hontanilla et al., 1998; Munkle et al., 2000;). In the central nervous system, the two proteins CB and PV occur mostly in the neuronal populations that are involved with specific calcium – dependent mechanisms. GABAergic neurons from different brain regions contain high levels of parvalbumin. Calcium-binding protein parvalbumin is preferentially associated with spontaneously fast-firing, metabolically active neurons and coexists with gamma-amino-butyric acid (GABA) in cortical inhibitory interneurons. Parvalbumin is a slow $Ca^{2+}$buffer that may affect the amplitude and time course of intra-cellular $Ca^{2+}$transients in terminals after an action potential, and hence may regulate short-term synaptic plasticity.

The protein CB occurs in the striatonigral pathway that undergoes degeneration in some of the neurodegenerative diseases (e.g., Huntington's disease). The protein CB is mainly involved in the intracellular calcium homeostasis.

Parvalbumin on the other hand, is only associated with fast - firing neurons. It helps specific neurons to maintain a fast-firing rate by reducing the $Ca^{2+}$ - dependent potassium outflow, responsible for 'after hyperpolarisation period'. Thus PV containing neurons have a high metabolic rate. PV containing interneurons are among the very few cells that can survive certain degeneration.

PV positive cells are present in the number of basal ganglia structures; striatum, globus pallidus and substantia nigra (Munkle et al., 2000)

## Heat shock proteins

Heat shock proteins (hsp) belong to an extensive family of proteins. Heat shock and related proteins are major component of the central nervous system response to metabolic stresses. They act as chaperones, the proteins that bind to and stabilise an otherwise unstable conformer of another protein. By controlled binding and releasing of the substrate under physiological conditions, hsp facilitate molecule's correct fate: folding, oligometric assembly and transport to a particular subcellular compartment, or controlled switching between active/inactive conformations, thus limit protoxicity (Hendrick and Hartl, 1993). Some members of the hsp family are induced and over expressed under stress conditions in a wide variety of systems including brain, and are thought to exert a protective function (Lindquist, 1986). Thus, induction of these proteins in the brain, have been interpreted as a marker of cellular injury (Gonzalez et al., 1989; Sharp et al., 1991).

grp75 is a mitochondrial hsp 70- family member of stress proteins in the rat brain (Massa et al 1995). grp75 has been implicated in transport of proteins across the mitochondrial membrane and their stabilization prior to final folding. grp75 is expressed following metabolic stress in rat brain and induced by a calcium ionophore and glucose deprivation. grp-75 is regulated in response to the altered activity in the indirect pathway, induced under stress condition such as lesions and is thought to exert a protective function .

When the degree of injury is small, induction occurs in the area of injury, similar to that for hsp70. But when the injury is extensive, grp75 is up regulated in the neurons outside the injury. The induction of grp75 may represent a sensitive marker of metabolically compromised tissue and drug related change in the brain.

grp 75 is highly expressed in the reticular and subthalamic nucleus, globus pallidus and amygdala.

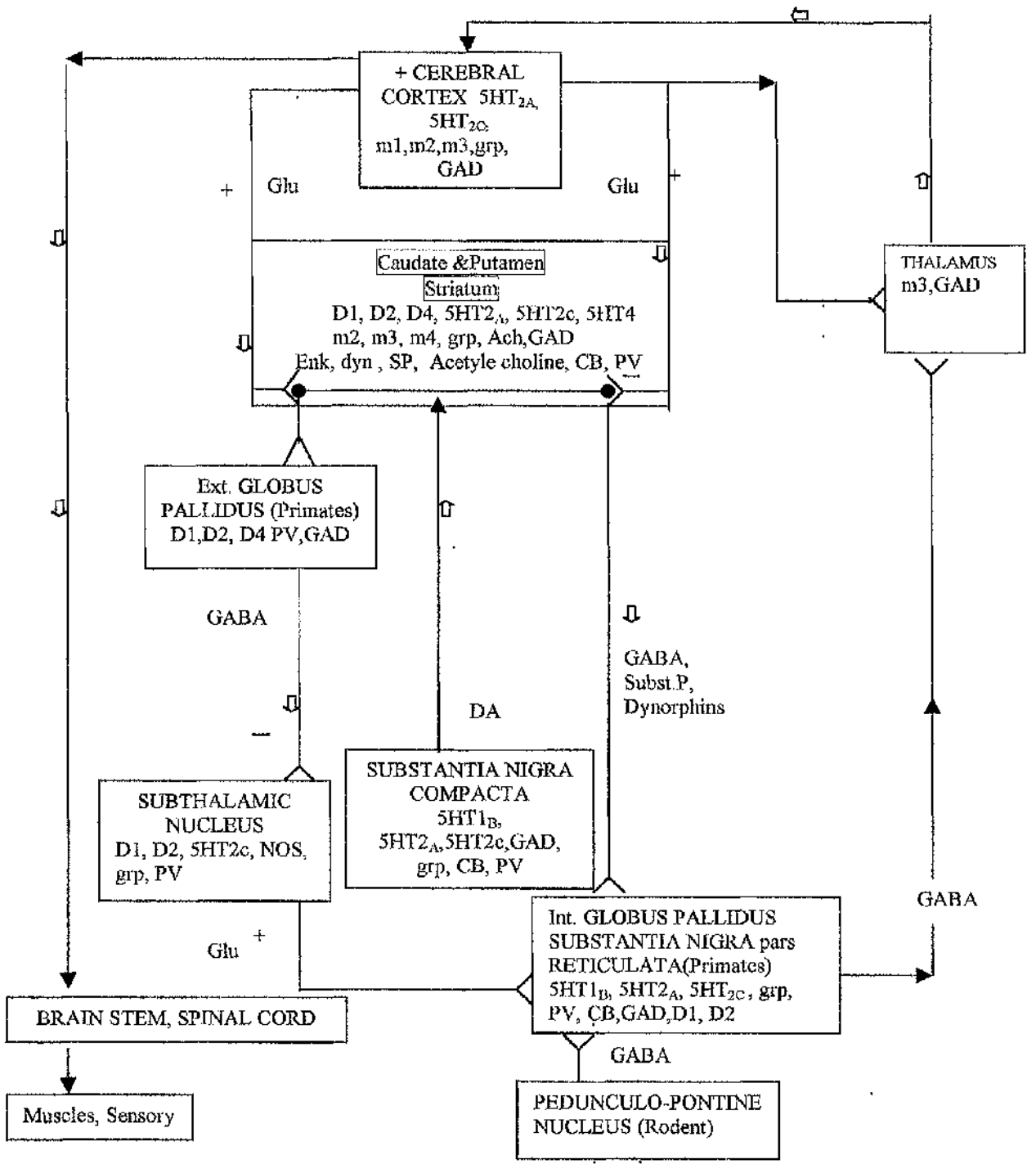


Fig.2. Schematic presentation of basal ganglion micro-circuitry with their neurotransmitters and neuropeptides.

## 1.1.7. Receptors in Basal ganglia

A diagram illustrating the location of different receptors in the basal ganglia is shown in Fig. 2 (page-46). Details of the various receptors and their subtypes are summarised in the following sections.

### Glutamate receptors

Glutamate receptors are classed into two groups: a) ionotropic receptors and b) metabotropic receptors.

There are three classes of ionotropic glutamate receptors: 1) N-methyl-D-aspartate (NMDA), 2) α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and 3) kainate. There are eight metabotropic glutamate receptors: mGluR1-mGluR8. These are again grouped into three groups: Group 1 (mGLu R1 & mGlu R5); Group 11 (mGlu R2 & mGlu R3); Group 111 (mGlu R4, mGlu R6, mGlu R7 & mGlu R8). Several types of mGluRs are located on the presynaptic terminals of central neurons.

### GABA Receptors

GABA receptors are divided into two main groups:
The ionotropic $GABA_A$ receptor is a heterooligomeric protein complex that consists of a GABA binding site coupled to an integral $Cl^-$ channel. $GABA_A$ is the site of action of anxiolytic benzodiazepines and other sedative hypnotics. They are considered as metabotropic.
In hippocampus, thalamus and cortex, low- intensity stimulation of inhibitory interneurons causes inhibitory postsynaptic potentials that are mediated entirely by $GABA_A$ receptors. $GABA_B$ receptors are expressed on both pre and post-synaptic membranes and like other $G_i$ linked receptors, open $K^+$ channels and decrease $Ca^{2+}$ conductances, and inhibit adenylyl cyclase.

Postsynaptic $GABA_B$ receptors produce a slower but longer lasting inhibition, but require much longer stimulation and / or higher frequency.

Low doses of GABA – mimetic stimulate presynaptic receptors. This would result in an anticholinergic effect, restoring the chemical balance of the striatum, and thus diminishing the EPS effects of antipsychotic drugs. High doses of GABA- mimetic would stimulate the postsynaptic GABAergic receptors, resulting in a blockade of dopaminergic transmission, and thus reinforcing the anti-dopaminergic effect of the neuroleptic and EPS. Thus modulation of glutamate (principal excitatory neurotransmitter) and GABA (principal inhibitory neurotransmitter) receptors is of paramount importance for many brain functions and dysfunction of the GABA system may be a major contributory factor in the pathology of schizophrenia (Ossowska et al., 1984).

## Dopamine Receptors

Molecular and pharmacological studies reveal that there are at least five distinct dopamine receptors: $D_1$, $D_2$, $D_3$, $D_4$, and $D_5$. All catecholaminergic receptors belong to the G-protein coupled receptor superfamily.

The dopamine receptors are classified into two main classes: 1) $D_1$ – like, consists of $D_1$ and $D_5$ and 2) $D_2$ – like, consists of $D_2$, $D_3$ and $D_4$ receptors. Both the two classes of receptors exert their biological activity by activating G-proteins after coupling with them. $D_1$ dopamine receptors interact with Gs complexes resulting in the activation of adenylate cyclase and an increase in intracellular cAMP levels. Whereas the $D_2$ dopamine receptor interacts with Gi complexes to inhibit cAMP production and considered not to be linked to the adenylate cyclase.

1) $D_1$ receptors are ten times more abundant in brain than $D_2$ like receptors. $D_1$ receptors are expressed predominantly by pyramidal neurons on their dendritic spines, which

are an ideal location for modulation of the glutamate-mediated inputs to this. $D_1$ mRNAs are also found in high levels in the caudate-putamen and nucleus accumbens.

2) $D_2$ receptors are present in very high numbers in caudate-putamen, the nucleus accumbens and substantia nigra. They are expressed in the pyramidal cells as well as in the GABA-containing interneurons (local-circuit neurons) and glutamate-containing nerve terminals that innervate the region. $D_2$ receptors are a major site of action of typical antipsychotic drugs.

   $D_2$ receptors often serve as autoreceptors because their activation can inhibit the cells in which they reside. Dopamine release from a nerve terminal can activate $D_2$ receptors present in the presynaptic terminal and can reduce the synthesis or release of more dopamine. Thus, $D_2$ receptor acts as a negative feedback mechanism to modulate or terminate dopamine signaling at a particular synapse.

   The current concept about functional organisation of the basal ganglia suggests a critical role for $D_2$ receptors and the indirect pathway in the pathophysiology of EPS. It had been shown that $D_2$ receptor-deficient mice lacking either one or two functional copies of the $D_2$ receptor showed deficit in movement initiation, although they have normal movement speed, no postural abnormalities and no tremor (Murer et al., 2000). There is a good correlation between antipsychotic activity and affinity for the $D_2$ dopamine receptor subtype for a wide range of dopamine receptor antagonists. The neuroleptics with less inhibitory action on the nigrostriatal dopamine system produce less cataleptic behaviour and cause much lower incidence of extrapyramidal symptoms. A selective deficit in $D_2$ receptor mediated dopamine actions could lead to the development of some specific neurochemical changes in the basal ganglia indirect pathway in the Parkinson like side effects of antipsychotic drugs.

3) $D_3$ receptors are found mostly in the Islands of Calleja and in the nucleus accumbens and cerebellum (Sokoloff et. al., 1992a).
4) $D_4$ receptors are found in frontal cortex, midbrain area, amygdala, hypothalamus, and thalamus.

5) $D_5$ mRNA is only present in the hippocampus, hypothalamus and paraphasicular nucleus of the thalamus.

All existing antipsychotics possess dopamine receptor antagonistic properties.

## Noradrenaline and adrenaline Receptors

As in all catecholaminergic receptors, these receptors also belong to G protein-coupled receptor super family.

Adrenergic receptors are divided into α and β categories, both of which comprise many receptor subtypes. Each subtype responds in varying degrees to both noradrenaline and adrenaline. All β receptors are $G_S$ –coupled, and most $\alpha_2$ receptors are $G_i$ –coupled, functioning not only as heteroreceptors but also as autoreceptors for catecholaminergic neurons. The $\alpha_1$ adrenoreceptor has also been suggested as a possible candidate involved in the atypical clinical profile of ADP like clozapine (Wadenberg and Hicks, 1999)

## Serotonin Receptors

Fourteen distinct serotonin receptors have been cloned so far, and are classified into 7 receptor families. Pharmacologically and functionally, there is greater understanding of 5-HT1, 5-HT2, 5HT3, and 5HT4 families than the 5-HT5, 5HT6 and 5HT7 subtypes. All 5HT receptor families are all G-protein coupled receptors with the exception of 5HT3 which is ligand gated ion channel coupled (Kinsey et al., 2001). 5-HT1 receptors couple with $G_I$ protein; 5-HT2 couple with $G_q$ protein; and 5-HT4, 5-HT6, 5-HT7 receptors are coupled to $G_S$ protein; coupling for 5-HT5 has not been determined.

The serotonergic 5-HT1A receptor is termed a somatodendritic autoreceptor because it resides on serotonergic cell bodies and dendrites. Its activation reduces cell firing and reduces the synthesis and release of serotonin. Serotonergic 5-HT1D (in human)

receptors and 5-HT1B (in rodents) receptors decreases the local synthesis and release of transmitter. 5-HT1A and 5HT1D receptors are highly homologous and both signal by coupling to $G_I$ proteins.

Out of all these 5-HT receptors 5-HT2 aroused interest amongst neuroscientists because of the high affinity of atypical antipsychotics for $5HT_{2C}$ receptors. This fact further suggests that this receptor may play a role in the antipsychotic activity of these agents and may have possible role in the action of atypical neuroleptic drugs.

Most of the atypical antipsychotic drugs, clozapine, risperidone, olanzapine, quetiapine and some other atypical neuroleptic drugs target other neurotransmitter receptors along with dopamine receptors, such as 5HT receptors (Barnes and Sharp 1999; Bymaster et al., 1996; Kapur et al., 1999; Meltzer, 1989,1991; Pilowsky et al 1992; Stockmeier et al 1993; Sebens et al 1998; Tandon, 1970; Wadenberget al., 1997). These compounds have considerable affinity for the subgroups of $5HT_2$ receptors such as $5\text{-}HT_{2A}$, $5HT_{2B}$ and $5HT_{2C}$. Amongst these, $5HT_{2C}$ have been suspected to have influence in the motor control and EPS. Affinity for $5HT_{2B}$ receptor for these drugs could be significant in producing EPS, but not very much is known about this yet.

These $5\text{-}HT_{2C}$ receptor is a prominent serotonin receptor systems, was found in the brain areas such as: basal ganglia (Wolf and Schutz 1997) substantia nigra (pars compacta and reticulata), globus pallidus, cerebral cortex (pyriform, frontal and cingulate cortex) and olfactory tubercle, nucleus accumbens, caudate-putamen, amygdala and thalamus. They are also found in raphe nuclei and in adjacent reticular nuclei, suggesting that $5HT_{2C}$ receptors may function as autoreceptors on serotonergic neurons (Eberle-Wang et al., 1997; Palacios et al., 1990; Pasqualetti et al., 1999; Pompeiano et al, 1994; Stefulj et al., 2000).

The $5HT_{2C}$ receptors are known to have both high and low agonist affinity states. It had been suggested that the interaction of serotonin with the low affinity state of $5HT_{2C}$

receptors are responsible for signal transduction. Activation of $5HT_{2C}$ receptors induces an increase in intracellular calcium and a calcium -induced $K^+$ current.

Although the physiological roles of 5-$HT_{2C}$ are not completely known, administration of $5HT_{2C}$ receptor agonists can influence locomotion, feeding behaviour, body temperature, hormone secretion, and produce anxiogenic effects (Kennett et al., 1989). This receptor has also been implicated in obsessive compulsive disorders and migraine (Curzon and Kennett, 1990).

## Acetylcholine receptors

Acetylcholine (Ach) receptor elicits its effects in the CNS by means of two different classes of receptor systems: a) nicotinic receptors, which are ligand-gated channels, and b) muscarinic receptors, which are from the G protein-coupled receptor superfamily.

The activation of nicotinic Ach receptors by Ach leads to the rapid influx of $Na^+$ and $Ca^+$ and subsequent cellular depolarisation.

All five muscarinic receptor subtypes (Caulfield and Birdsall, 1998) are expressed in the brain, and can be subgrouped based on their patterns of G protein coupling; M1, M3, and M5 receptors couple with $G_q$ proteins. M2 and M4 receptors couple to $G_i$ proteins (Birdsall et al., 1983; Burstein et al., 1997). M1, M3and M5 receptors produce their effects by stimulating or inhibiting activation of the phosphatidylinositol system. As like most $G_i$ –linked receptors, M2 and M4 receptors elicit mostly inhibitory responses. They act by activating inwardly rectifying $K^+$channels, inhibiting voltage gated $Ca^+$ channels, and inhibiting adenylyl cyclase (Zhang and Bymaster, 1999). M1, M2, M3, and M4 receptors are present in the substantia nigra. M1, M3 and M4 receptors are located in the cerebral cortex and hippocampus, where their activity may mediate some of the effects of

Ach on learning and memory (Felder, 2000; Eglen et al., 2001). M1 and M4 subtypes are believed to mediate cholinergic signaling in extrapyramidal motor circuits. M2 receptors, which are least abundant, are concentrated in the basal forebrain, the site of several cholinergic nuclei. It is believed that they may act as autoreceptors to control Ach synthesis and release from forebrain cholinergic neurons. M5 receptors are least abundant of the muscarinic receptors and are scattered through out the brain.

## Histamine Receptors

Three histamine receptors have been identified; $H_1$, $H_2$, $H_3$. All are G- protein coupled receptor superfamily. $H_1$ couple to $G_q$ proteins and $H_2$ receptors couple to $G_s$ proteins; $H_3$ coupled to $G_i$ proteins and may act as autoreceptors, functionally analogous to the α-adrenergic, dopamine $D_2$ and 5-$HT_1$ receptors. The $H_3$ receptor also functions as heteroreceptors and regulates the release of other neurotransmitters from nerve terminals. Many drugs that cause drowsiness contain $H_1$ receptor antagonists as active ingredients, which can cross blood brain barrier. Newer medications are non sedating because they do not cross the blood-brain barrier. Olanzapine and Y931 also have high affinity for $H_1$ receptors.

## Neuropeptide Receptors

There are many neuropeptide receptors. Some neuropeptides binds with only one known receptor, while others bind to more than one receptor subtypes. Similarly, some neuropeptide receptors have the ability to bind more than one peptide.

The presence of neuropeptide receptors in the basal ganglia parallels the presence of the corresponding neuropeptides, with high levels of opioid, tachykinin, somatostatin and NPY receptors, plus many others at lower levels.

## 1.1.8. INVESTIGATION OF THE ANOMALOUS LOW INCIDENCE OF MOTOR SIDE EFFECTS OF THE COMPOUND – Y931

The compound Y-931 {8-fluoro-12- (4-methylpiperazin-1-yl)-6H-[1] benzothieno [2,3-b][1,5] benzodiazepine maleate} is a new potential atypical antipsychotic drug (Morimoto et al., 2000). It is classed as a multi-affinity receptor target agent similar to clozapine and olanzapine. Y-931 interacts with receptors such as dopaminergic, serotonergic, α-adrenergic, muscarinic and histaminergic receptors. In models of N-methyl-D-aspartate (NMDA) receptor activity, Y-931 demonstrated the most potent protective action against the dizocilpine-induced neurotoxicity (neuronal vacuolization) in the rat retrosplenial cortex (Morimoto et al., 2002). It reportedly gives very few (if any) extra pyramidal side effects (EPS) in animal models at equivalent doses (MPC unpublished data).

Y931 has a similar receptor - profile to clozapine and olanzapine, that is, high affinity for $D_2$, 5-$HT_{2c}$, 5-$HT_6$, 5-$HT_7$, α-Adreno-receptors, mACh, muscarinic receptors M1, M2, M3, M4, M5, and Histamine H1 (MPC unpublished data). The overall aim of this study was to investigate the mechanisms responsible for the lack of EPS during Y931 treatment, despite significant affinity of the drug for $D_2$ receptors.

Three hypotheses can be suggested, based on the current knowledge of Y-931 pharmacology, and on the premise that Y-931 blocks striatal $D_2$ sites in sufficient levels to cause EPS, but also acts at other sites in the CNS to prevent the expression of EPS.

1) Y-931 acts within the striatum to suppress the activation of the indirect pathway, despite blocking striatal $D_2$ dopamine receptors. The antagonist actions of Y-931 at muscarinic receptors in the striatum could be involved, acting to suppress the EPS via the suggested interaction between muscarinic and $D_2$ dopamine receptors in the striatum.

2) Y-931 acts outside the striatum to suppress the activation of the indirect pathway. Via its relatively high affinity for 5-$HT_{2C}$ or muscarinic M3 receptors, Y-931 may act to suppress EPS at a site downstream of the striatum. Muscarinic M3 receptors increase STN activity, so antagonism at this receptor may act to suppress the abnormal stimulation of the indirect pathway following blockade of striatal $D_2$ receptors. Also, $5HT_{2C}$ receptor antagonism suppresses STN activity, so the $5HT_{2C}$ receptor antagonist properties of Y-931 may be involved in reducing EPS.

3) Y-931 acts to elevate the activity of the direct pathway. Y-931 may facilitate stimulation of nigrally projecting neurons in the striatum by the motor cortex via a facilitation of glutamatergic NMDA receptor activation. The direct pathway from the striatum to the SNr could show increased activity in response to Y-931 relative to the indirect pathway.

Neurochemical markers of neuronal activity (immediate early genes and GABA neuron markers) will be employed for the analysis of above hypothesis. In addition, the role of $5HT_{2C/2B}$ receptors in modulating the neurochemical changes and in modulating haloperidol-induced catalepsy will be investigated.

## 1.1.9. Aims of this thesis

In the studies presented in this thesis, the aim is to compare the regional neurochemical effects of Y931 with olanzapine, an atypical antipsychotic drug and with the conventional antipsychotic drug haloperidol. This will enable the determination of any distinctive characteristics of Y931 in the basal ganglia-thalamocortical neuronal pathways, to explain its anomalous behaviour in exhibiting very few (or, any at all) EPS.

# CHAPTER 2: Comparison of the effect of acute treatment with haloperidol, Y931 and olanzapine on immediate early gene expression and some of the marker genes of the motor circuits in the rat basal ganglia.

## 2.1. Introduction

### Effect of acute treatment with haloperidol and Y931 on immediate early gene (IEG) expression and some of the marker genes of the motor circuits in rat basal ganglia: Study of Immediate Early Gene expression to characterise Y- 931.

Immediate early genes are a unique class of genes. Transcription of these molecules can be activated within minutes and characteristically their induced transcription is very short-lived. They are also expressed at unusually low levels basally. The c-fos and c-mycoproto -onco-genes were some of the immediate early genes that were first discovered (Greenberg and Ziff 1984). The rapid accumulation of these products combined with histological methods that offer detection at the cellular level are key features that have led to their wide use in visualizing activated neurons.

Many IEGs have been identified in the brain. They are divided into families depending on their structural similarities. The members of fos- or jun families are closely related to c-fos or c-jun. There are also some IEGs, which are structurally unique, such as zif-268, a zinc finger containing protein (Millbrandt, 1987).

Immediate early genes (IEGs) act as transcription regulatory factors by coordinating changes in gene expression as they encode transcription factors. IEG induction has been widely investigated in relation to the mechanism of action of antipsychotic drugs (Sebens et. al., 1998). Inducible transcription factors, including certain immediate early genes, may mediate between receptor-activated second messenger systems and expression of genes involved in the differentiated functions of neurons.

All clinically effective APDs have high affinity for dopamine receptor, specifically for $D_2$ receptors. c-fos is believed to be an initiator of several biological adaptations. And all clinically effective APDs induce the IEG c-fos and its protein product Fos in the forebrain of mammals with a characteristic regional pattern of expression. Typical and atypical

antipsychotic drugs are known to elevate the level of certain IEGs in certain areas of brain. Antipsychotic drugs with dopamine $D_2$ antagonist properties, such as haloperidol, induce both c-fos and zif 268 mRNA in caudate-putamen. But, atypical antipsychotic drugs, e.g., clozapine, induce zif268 but not c-fos mRNA in that region (Nguyn et al., 1992). After treatment with specific antipsychotic drugs, such as haloperidol, olanzapine and Y931, the expression level of zif 268, c-fos and fra-2 mRNA (the most investigated IEGs) in different areas of brain should give an idea, how differently these APDs act in different areas of brain. And how Y931 could modulate the activity of direct and indirect pathways in basal ganglia through which it could ameliorate EPS.

Both the Fos and Jun family of nuclear phosphoproteins may group together to form homo - or heterodimeric AP-1 (activator protein–1) complexes. This complex (AP–1) then activates protein kinase C to mediate transcriptional responses. The content of AP-1 may vary under different physiological conditions. Closely related members of the same family may contribute to quite distinct biological phenomena. These complexes then bind to a DNA consensus sequence that is known to be present in numerous gene promoters. After binding to the gene, AP-1 may either activate or repress the candidate gene. Thus AP-1 complexes are indispensable in the regulation of neural gene expression by extracellular signals.

Zif 268 belongs to the zinc -finger class of transcription factors. The protein contains finger-like protrusions that are stabilised by ionic zinc, which act directly on the regulatory sequence GCGG/TGGGCG. The presence of zif268 at the promoter site serves to modulate the expression of the target gene. Thus both the c-fos and zif268 act as third messengers in activating the target genes.

fra-2 is a late expressed fos related IEG gene found mainly in the cerebral cortex and in thalamus. fra-2 mRNA is expressed later than c-fos and zif-268 and persist long after most of IEG mRNA expression is lost. It had been shown that dopamine lesions induce a prolonged expression of Fos-related antigens (FRA's) in the striadopallidal neurons, which are known to contain $D_2$-dopamine receptors. FRA's are members of the leucine-zipper family of transcription factors that regulate neuronal gene expression. FRA's must

dimerise with the Jun-family for DNA-binding activity. $D_2$-dopamine receptor antagonists also induce FRA's in the striatal neurons (Dragunow et. al., 1995).

Some studies have shown that glutamate binding to the NMDA receptor can induce both c-fos and zif268. It has also been reported that the opening of the voltage sensitive calcium channels could trigger c-fos expression after activation of AMPA-kainate receptors by glutamate. In either condition ionic calcium is recognised as a major mediator of induction of both AP-1 transcription factors and zif268 gene (Robertson and Fibiger, 1996).

In the present studies, the pattern of gene activation of c-fos, fra-2, zif-268- member of immediate early genes and GAD67 (Glutamic acid decarboxylase 67), grp-75 (glucose regulating protein, a relation of heat shock protein group hsp-72), NOS (Nitric oxide synthase) and parvalbumin - some of the marker genes of motor circuits in basal ganglia, will be investigated using in-situ hybridisation. After treatment with Y931, haloperidol and other drugs, in situ hybridisation may reveal the regional sites of activity of these drugs and Y931, from which we may be able to gain information on how Y931 could work giving less or no extrapyramidal side effects.

A potential drawback of using in-situ hybridisation is, the experimental animals need to be carefully treated to have minimal stress during the procedure, as this may cause IEG expression. But, with appropriate controls, IEG induction should provide good indication of the brain regions affected by the drug treatment.

## 2.2.Aim.

The aim of the studies presented in this chapter, was to determine whether haloperidol, Y931 and olanzapine produce distinct patterns of mRNA expression of immediate early gene (IEG) expression and expression of NOS, GAD 67 and grp 75 mRNAs in the different regions of rat brain.

## 2.3. Material and Methods

### Drug treatment:

| Name of the drugs | Source | Dosage |
|---|---|---|
| 1. Haloperidol | RBI | 1 mg / kg |
| 2. Y931 | Welfide Corporation | 10 mg / kg |
| 3. Vehicle [0.5% (HPMC)] | Welfide Corporation | 1ml / kg |

Table 4. Drugs used for the acute treatment study.

24 male hooded Long Evans rats (235-295g) were purchased from Harlan-Olac, UK. Animals were housed under a 12 hour light /dark cycle with free access to food and drink. Animals were divided into three groups at random. Eight animals were in each group.

Group A = Vehicle (0. 5%HPMC)

Group B = haloperidol (1 mg / kg)

Group C = Y931 (10 mg / kg)

On the day of treatment, the animals were injected intra-peritoneally (i.p.) with vehicle 0.5% hydroxypropylmethylcellulose (HPMC), 1ml/kg of body wt., haloperidol (1mg / kg) or Y931 (10 mg / kg). We are using Y931 at this dose because of its antipsychotic property such as inhibition of apomorphine induced hyperactivity and suppression of conditioned avoidance response (CAR) at this dose (Morimoto at al., 2002). Animals were randomly

allocated to treatment groups. Forty-five minutes after injection each rat was killed by decapitation. Brains were quickly removed and frozen in iso-pentane cooled to – $42^0$C on dry ice. Brains were coated with embedding medium (Lipshaw) and stored at –$70^0$C until required.

All animal experiments were carried out in accordance with the U. K. Animals (Scientific Procedures) Acts, 1986 and related guidelines.

20 μm cotronal sections were cut from four different levels of each brain. Sections for each level were taken according to ' The Rat Brain' atlas (Paxinos and Watson, 4th. Edition. 1995). Each level was as follows:

Level 1 (Plate-12): visualising- 1) accumbens shell and core, 2) cingulate cortex area 1&2, 3) primary motor cortex and secondary motor cortex and 4) caudate putamen (striatum).

Level 2 (Plate-24): visualising- 1) somatosensory cortex (area 1 and 2), 2) caudate putamen and 3) lateral globus pallidus.

Level 3 (Plate 36): visualising- 1) retrosplenial agranular cortex, 2) subthalamic nucleus, 3) para subthalamic nucleus.

Level 4 (plate 42): visualising- 1) substantia nigra pars reticulata, 2) substantia nigra pars compacta, 3) substantia nigra, lateral part.

The above areas are displayed in fig.3. (Page: 63-64)

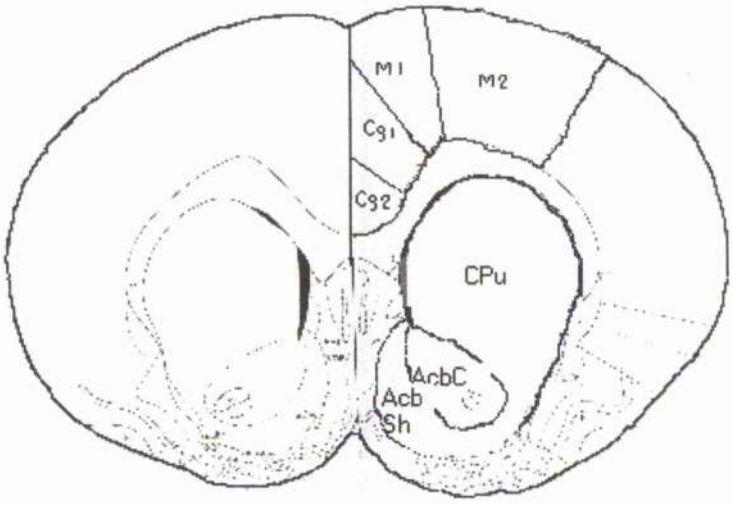


Plate –12 (Paxinos & Watson, 4th Edition 1998): showing striatum (Cpu), Accumbens Core & shell (AcbC & AcbSh), Cingulate cortex- 1and Cingulate cortex-2, Motor Cortex-1 and motor cortex-2.

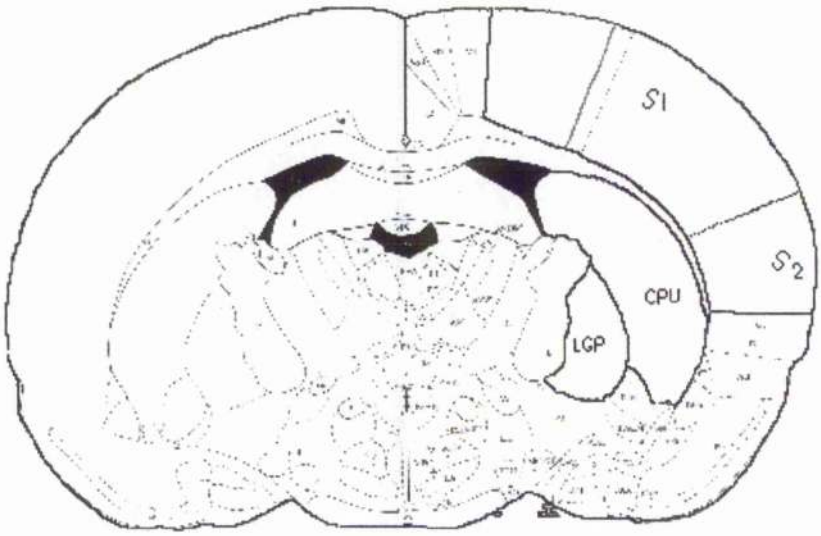


Plate –24 (Paxinos & Watson, 4th Edition 1998): showing striatum (Cpu), Lateral globus pallidus (LGP), Sensory Motor cortex-1 and 2 (S1, S2)

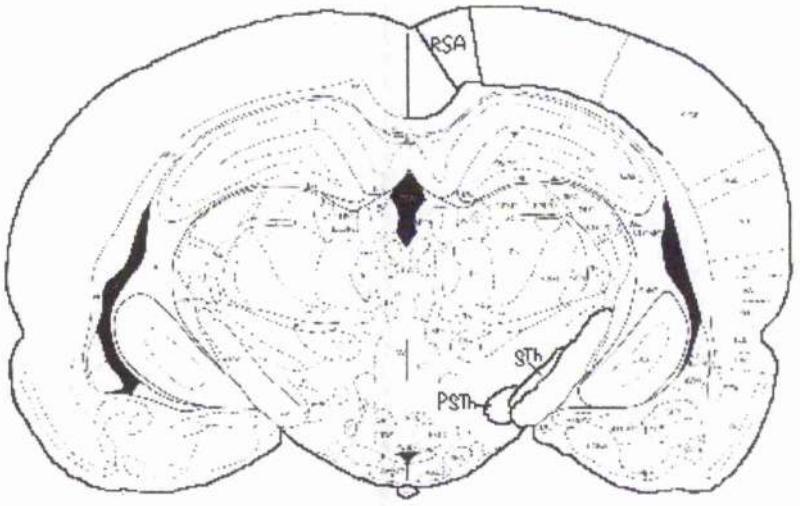


Plate –35 (Paxinos & Watson, 4th Edition 1998): showing subthalamic nucleus (STh / STN) and retrosplenial cortex, agranular (RSA).

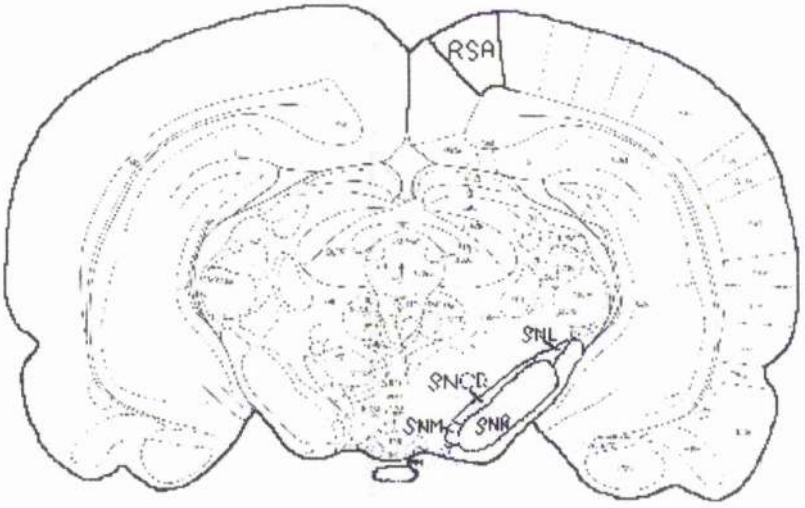


Plate –42 (Paxinos & Watson, 4th Edition 1998): showing –Substantia nigra pars reticulata (SNR), Substantia nigra pars compacta (SNCD).,Substantia nigra, Lateral part (SNL), Retrosplenial cortex, agranular (RSA).

Fig: 3. Schematic representation of brain levels in which sections were collected for in situ hybridisation (taken from Rat Brain in stereotaxic co-ordinates, 4th Edition, Paxinos and Watson 1998).

Two sections from the same level of each brain and sections from two animals were placed on one poly-L-lysine coated slide (previously baked at 180$^{O}$ C and coated in a 0.01%poly-L-lysine solution made up in DEPC treated water). Slides were labelled appropriately. Some sections were also cut from control animal brains at the same levels for use as controls. A LEICA CM1850 cryostat at –20$^{0}$ C was used for cutting the sections. Sections were dried at RT then fixed briefly in freshly prepared 4% (w/v) paraformaldehyde in phosphate buffered saline (PBS). After rinsing, the sections were sequentially dehydrated in 70%, 95% and absolute alcohol and then stored under absolute alcohol at 4$^{0}$C.

## 2.3.1. In situ hybridisation (Wisden and Morris, 1994)

In-situ hybridisation is used for tracing the regional sites of gene expression in the tissue. A radiolabelled nucleotide tagged nucleic acid probe is allowed to hybridise directly to a tissue section, where it recognises its cognate mRNA and forms a probe – mRNA complex. Excess probes are then washed off and tissue section is then exposed for autoradiography to reveal the sites on the tissue section, treated by the probe.

There are many different types of probes, e.g., Oligonucleotides, cDNA probes, cRNA probes. Oligonucleotides are most commonly used probe as different oligonucleotides could be distinguished easily differing in small regions of nucleotide sequences in their mRNA. The technique for using this probe is simple and does not need very much previous molecular biology experience.

Oligonucleotide probes of unique sequence were used for in-situ hybridisation (ISH). These were:

1) zif-268 (45 mer) 5′ CCG TTG CTC AGC AGC ATC ATC TCC AGT TTG GGG TAG TTG TCC 3′
Complementary to nucleotides spanning amino acids sequence 2 – 16 (Millbrandt, 1987)

2) c-fos (45 mer) 5′ CTG CAG CCA TCT TAT TCC TTT CCC TTC GGA TTC TCC GTT TCT CTT 3′
(Curran et al., 1987)

3) GAD 67 (45 mer) 5′ GCA TCC ATG GGC TAC GCC ACA CCA AGT ATC ATA TGT TGT AGG ACG 3′

4) NOS (45 mer) 5′GGC CTT GGG CAT GCT GAG GGC CAC TAC CCA GAC CTG TGA CTC TGT 3′

All the above oligonucleotides were purchased from GenBank, UK.

4) grp 75 (45 mer) 5′ AAC AAC TGC ACC CTT GAT TGC TTC TGA CGC ATA ATC CCT TCT TGA 3′

The above probe was purchased from Thermo Hybaid GmbH, Germany.

## Labeling of oligonucleotide probe:

Probes were end-labelled with deoxynucleotide transferase (TdT) in presence of $^{35}$SdNTP (46.3 MBq / mmol) in TdT buffer. Molar ratio of $^{35}$SdATP to oligonucleotide was 30:1.

| Name of the reagent | Source |
|---|---|
| 1. Isopentane | Fisher Chemicals, Loughborough UK. |
| 2. Terminal deoxyneucleotidyle Transferase (TdT enzyme) | Boehringer Mannheim, Lewes UK. |
| 3. TdT tailing buffer | Amersham pharmacia Biotech, Herts UK. |
| 5. Diethyl pyrocarbonate (DEPC) | Sigma, Poole UK. |
| 6. Dithiothreitol (DTT) | As above |
| 7. Paraformaldehyde | As above |
| 8. Polyadenylic acid | As above |
| 9. Sodium Phosphates | British Biochemicals International (BDH) |
| 10. Sodium pyrophosphate | As above |
| 11. Glacial acetic acid | As above |
| 12. Dextran sulphate | Amersham Life Sciences UK. |
| 13. Formamide | Fluka, Poole UK. |
| 14. α-[ $^{35}$S ]dATP | New England Nuclear, Hounslow UK |
| 15. Qiagen QIAquick Nucleotide Removal kit | Qiagen Ltd, Sussex UK |
| | |

Table 5. Reagents for labelling Probes and in situ hybridisation. All the stock solutions for in situ hybridisation were made and autoclaved / sterile filtered and kept in small aliquots at the appropriate temperature.

This reaction initiates the reaction for the formation of single stranded DNA from the 3′ terminal. Reaction mix for this was as shown in the table 6.

| | |
|---|---|
| DEPC $H_2O$ | 0.6 µl |
| TdT tailing buffer | 1.2 µl |
| Oligonucleotide | 1.5 µl |
| $^{35}$SdNTP | 1.5 µl |
| TdT enzyme | 0.6 µl |
| Total vol. | 5.4 µl |

Table 6. Reaction mix for labelling

The mixture was incubated in a $37^0$ C water bath for 1 hour. The reaction was then terminated by adding 60 µl of DEPC treated water.

To remove the un -incorporated nucleotides, Quagen QIAquick Nucleotide Removal kit was used according to manufacturer's instructions. The extent of probe labelling was assessed using β-scintillation counting. Probes labelled from $10^6$ to $3x10^6$ d.p.m / µl $^{-1}$were used for in situ.

Labelled probe was then stored at $-20^0$ C after adding 2 µl 1M DTT.

On the day of the in situ hybridisation experiment, sections were removed from absolute alcohol and allowed to air dry for 30 mins. Then the sections were hybridised with selected probes as follows:

The radiolabelled probe was diluted 1:100 with hybridisation buffer [50% deionised formamide, 2% 5mg/ml polyadenylic acid, 20% 20x standard saline citrate (SSC), 5% 0.5M sodium phosphate pH 7.0, 1% 0.1M sodium pyrophosphate and 10% dextran sulphate made up to 50 ml with DEPC-treated water]. After adding DTT 4:100 mixture was vortexed thoroughly. 200µl of this mix was applied to each slide.

For control hybridisation, a 50 folds excess of unlabelled "cold" probe was added to test for displacement of the specific signal.

| Solution added | Control mix | Test mix |
|---|---|---|
| Hybridisation buffer | 200 µl | 200 µl |
| 1 M DTT | 8 µl | 8 µl |
| Hot Probe | 2 µl | 2µl |
| Cold Probe (5 ng / µl stock soln) | 16 µl | - |

Table 7. Hybridisation mixture for in situ hybridisation.

The slides were covered with parafilm. Any air bubbles were removed carefully. Four of these slides were placed carefully in a petri dish. A piece of tissue paper soaked in 4xSSC was placed carefully in one side in the petri dish to maintain humidity. The slides were incubated over night at $42^0$C.

The next day slides were transferred into glass troughs containing 250ml 1xSSC. The parafilm cover slips were gently removed. The slides were then washed in 1xSSC at $60^0$ C for 30 mins. with constant agitation. Following this, the slides were briefly washed at RT in1xSSC and then 0.1xSSC and then dehydrated through 70%, 95%and then in absolute alcohol, all at RT. Slides were then allowed to air dry.

The sections were exposed to autoradiographic film (Biomax MR film, Kodak) at room temperature for a week to five weeks, depending on the probe used and the efficiency of their tailing. The films were developed using a Kodak Hyperprocessor according to the manufacturer's instructions.

## 2.3.2. Data analysis

MCID image analysis system was used for quantification of IEG expression. Relative optical density (ROD) measurements were taken from duplicate sections from each animal. For each probe, individual one-way ANOVA was carried out for each discrete brain region followed by LSD Post hoc analysis to determine statistical significance of any drug induced changes in IEG expression.

## 2.4. Results

### 2.4.1. Results of in situ hybridisation of immediate early gene expression after acute treatment with antipsychotic drug treatment.

#### Effect of haloperidol and Y931 on zif 268 mRNA expression

Haloperidol (1mg/kg i.p.) and Y931 (10mg./kg. i.p.) increased zif268 mRNA expression in the most areas of the brain examined. Haloperidol produced significant increases in zif 268 mRNA expression levels in most of the regions of the brain measured, with the exception of the accumbens shell, and the motor cortex layer 1 (M1L1), layer 2 (M2L1). The most marked increases occurred in the dorsolateral caudate putamen and the ventromedial caudate putamen where increases in zif 268 mRNA expressions level were 196 % and 172% respectively compared to vehicle controls.

In contrast, Y931 produced a more restricted pattern of change in zif 268 mRNA expression. However, as with haloperidol there was a pattern of change in the caudate putamen and the core of the accumbens where the zif 268 mRNA expression level was significantly high.

Unlike haloperidol, Y931 failed to induce increases in zif 268 expression in the motor cortex (Table 8 page-72; Fig: 4 –8, page: 75 -78).

| Brain region | Veh | Haloperidol | | Veh/Y931 | |
|---|---|---|---|---|---|
| | ROD | ROD | % control | ROD | % Control |
| M1 Layer 1 | 0.291±0.230 | 0.365±0.020 | 125.4±6.9 | 0.310±0.020 | 107.8±6.9 |
| M1Layer 2 | 0.255±0.020 | 0.305±0.110 | 119.6±4.3* | 0.260±0.010 | 101.6±4.6 |
| M2Layer1 | 0.321±0.020 | 0.408±0.010 | 127.3±4.1 | 0.338±0.020 | 105.4±6.2 |
| M2Layer2 | 0.232±0.054 | 0.294±0.060 | 126.3±4.1* | 0.171±0.070 | 73.5±32.0 |
| Cg 1 | 0.388±0.020 | 0.498±0.020 | 128.2± 5.2* | 0.41±0.030 | 105.6±7.3 |
| Cg 2 | 0.273±0.020 | 0.348±0.015 | 127.7± 5.7 | 0.296±0.020 | 108.0±7.6 |
| DLCPu | 0.149±0.010 | 0.293±0.010 | 196.0± 8.7* | 0.319±0.030 | 213.3±20.6* |
| VMCPu | 0.187±0.020 | 0.323±0.010 | 172.5± 6.2* | 0.296±0.020 | 157.8±11.5* |
| AcbC | 0.103±0.010 | 0.153±0.010 | 149.0±10.2* | 0.155±0.010 | 149.5±9.0* |
| AcbSh | 0.116±0.010 | 0.152±0.010 | 131.0±10.2 | 0.161±0.020 | 139.0±20.0 |

Table 8. Effects of acute haloperidol (1 mg/kg) and Y931 (10 mg/kg) on zif268 mRNA induction in cortical (Motor cortex-1 Layer1, Motor cortex-1 Layer2; Motor cortex 2 Layer1, Motor cortex 2 Layer2), striatum (DLCPu, VMCPu, RSA) and nucleus accumbens (AcbC, AcbSh) brain regions. Data shown as mean optical density (ROD)± SEM and as mean percentage of vehicle control=100%, n=8 per group). Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate. *p<0.05 compared to respective vehicle control. # P<0.05 compared to haloperidol.

## Effect of haloperidol and Y931 on c-fos mRNA expression

The effect of acute treatment of haloperidol (1mg/kg i.p.) and Y931 (10 mg/kg) on c-fos mRNA expression are shown in the Table 9 and Fig (4 – 8). The acute administration of haloperidol produced a significant increase in the c-fos mRNA expression in regions of the rat brain measured with exception of motor cortex-1, the accumbens shell and core and retrosplenial cortex layer-1. As with the zif 268 mRNA expression level, the highest increases in c-fos mRNA expressions were in caudate putamen, both ventral and dorsal caudate putamen. But in contrast to zif-268 mRNA expression, the accumbens core did not show significant increase in c-fos expression level after treatment with either of the drugs. In some other cortical regions, haloperidol significantly increased c-fos mRNA expression, such as retrosplenial cortex (RSA L2) and the somatosensory cortex (S1L1 and S1 L2).

The most significant increase of c-fos mRNA expression occurred following the acute treatment of Y931 in the dorsolateral caudate putamen. In contrast to the zif268 expression level, the ventral region of caudate putamen did not show a significant increase in c-fos expression levels after treatment with Y931. However there was a similar percentage increase (≅60%) in both cases. But the expression level of c-fos mRNA, after treatment with Y931 was significantly higher than that of haloperidol in the dorsolateral caudate putamen, as well as in comparison to that of the controls (Table –9, page-74 and fig: 4-8, page: 75 - 78).

| Brain region | Veh/Veh | Veh/Hal | | Veh/Y931 | |
|---|---|---|---|---|---|
| | ROD | ROD | % Control | ROD | % Control |
| Cg1 | 0.105±0.010 | 0.155±0.0140 | 148±13 * | 0.112±0.0120 | 115±11 |
| Cg2 | 0.08±0.010 | 0.116±0.0120 | 142 ±14 | 0.0964 ±0.008 | 118 ± 9 |
| M1L1 | 0.048±0.003 | 0.069±0.008 | 143 ± 16 | 0.062±0.0170 | 128 ± 34 |
| M1L2 | 0.05±0.003 | 0.074±0.007 | 144 ±12 | 0.053±0.010 | 103 ±19 |
| M2L1 | 0.053±0.004 | 0.076±0.007 | 143 ±14 | 0.059±0.010 | 112 ± 19 |
| M2L2 | 0.053±0.004 | 0.075±0.007 | 141 ± 13 * | 0.065±0.004 | 122 ± 8 |
| VMCPu | 0.046±0.004 | 0.095±0.016 | 209 ± 35* | 0.076±0.014 | 165 ±30 |
| DLCPu | 0.03±0.003 | 0.134±0.011 | 430 ±35* | 0.209±0.041 | 670 ±130*# |
| AcbSh | 0.095±0.015 | 0.105±0.022 | 110 ± 23 | 0.053±0.012 | 55 ± 12 |
| AcbC | 0.06±0.012 | 0.044±0.005 | 73 ±8 | 0.038±0.009 | 63 ± 14 |
| RSA L1 | 0.118±0.013 | 0.082±0.014 | 69 ± 12 | 0.080±0.008 | 67 ± 6 |
| RSA L2 | 0.115±0.076 | 0.077±0.008 | 65± 7* | 0.067±0.009 | 58 ± 4* |
| S1 L1 | 0.175±0.024 | 0.115±0.016 | 65± 9* | 0.102±0.008 | 58± 4* |
| S1 L2 | 0.128±0.011 | 0.083±0.007 | 64±5* | 0.078±0.006 | 60 ±4* |

Table 9. Effect of acute haloperidol and Y931 on c-fos mRNA induction in cortical (Motor cortex-1 Layer-1and Layer-2; Motor cortex 2 Layer-1 and Layer-2), Cingulate cortex (Layer-1, Layer-2), retrosplenial Cortex (Layer-1, Layer-2), striatum (DLCPu, VMCPu) RSA), Somatosensory cortex (Layer-1 and Layer-2) and nucleus accumbens (AcbC, AcbSh) brain regions. Data shown as mean optical density (ROD) ± SEM and as mean percentage of vehicle control (control=100%, n=8 per group). Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate. *P<0.05 compared to respective vehicle. # P< 0.05 compared to haloperidol.

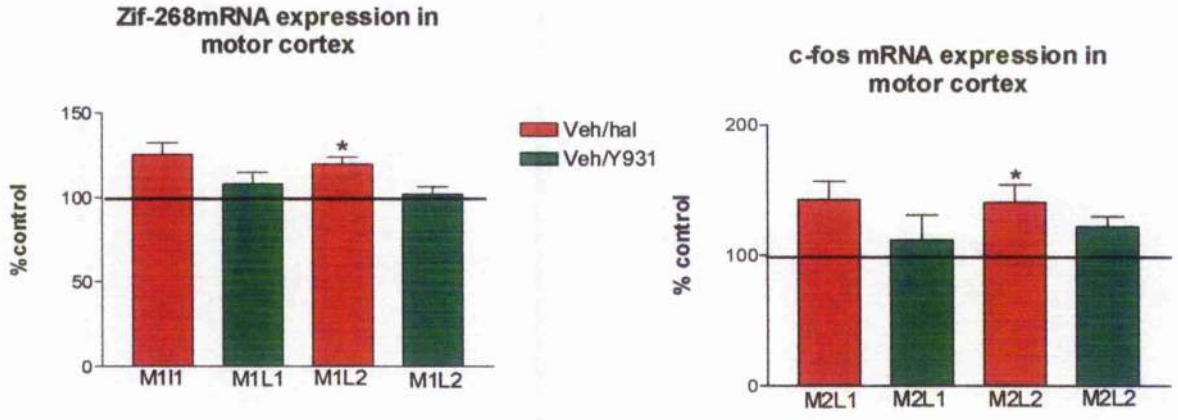


Fig.4. Effect of acute haloperidol (1mg/kg i.p.) and Y931 (10mg / kg i.p.) on zif268 and c-fos mRNA expression in the motor cortex, 45min after antipsychotic drug administration. Data represent mean % control ± SEM (n=8) analysed using one way ANOVA followed by LSD Post hoc analysis *P<0.05 compared to respective vehicle. # P< 0.05 compared to haloperidol.

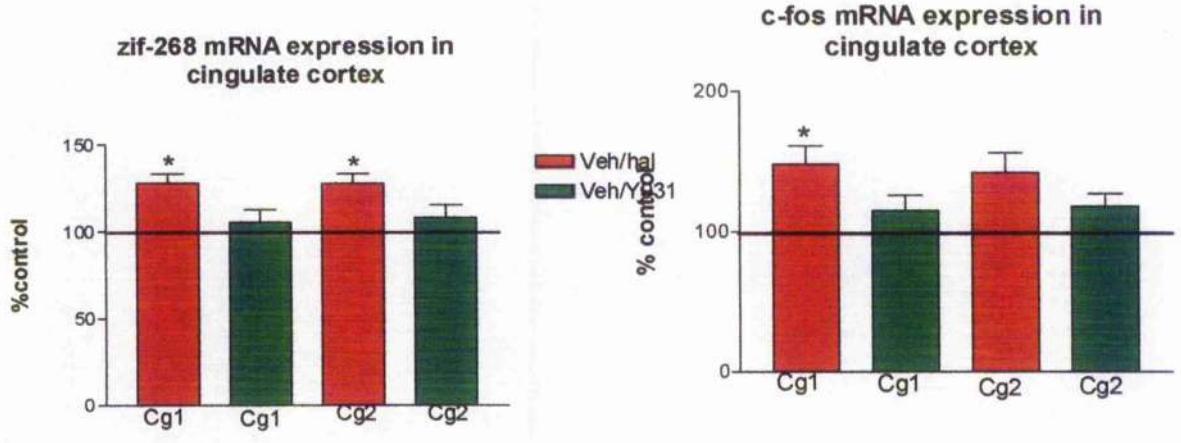


Fig.5. Effect of acute treatment of haloperidol (1mg/kg i.p.) and Y931 (10 mg/kg i.p.) on zif268 and c-fos mRNA expression in the cingulate motor cortex (Cg) areas after 45min. of antipsychotic drug administration. Data represent mean % control ± SEM (n=8) analysed using one way ANOVA followed by LSD Post hoc analysis *P<0.05 compared to respective vehicle.

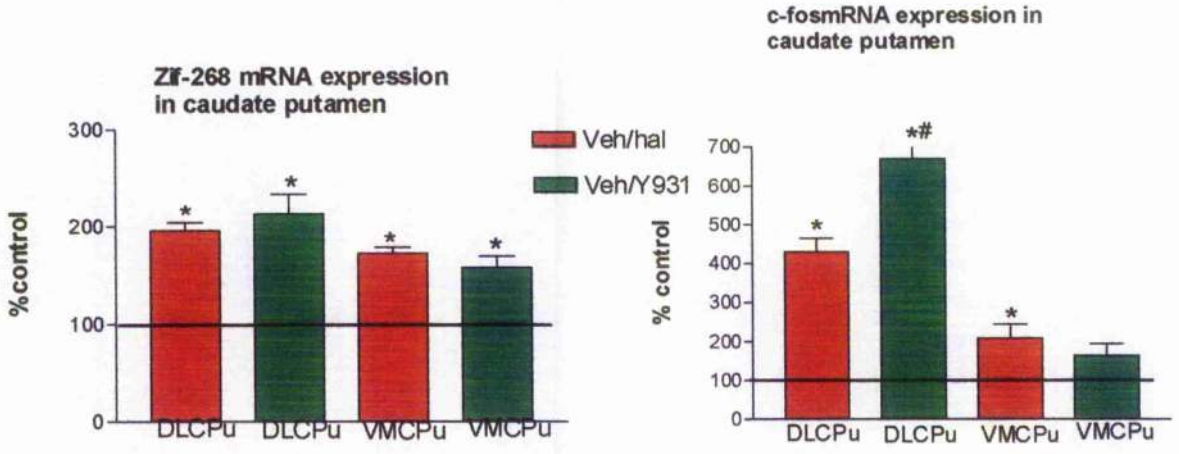


Fig.6. Effect of acute treatment of haloperidol (1mg/kg i.p.) and Y931 (10 mg/kg i.p.) on zif268 and c-fos mRNA expression in dorsolateral and ventro-lateral caudate putamen (DLCPu and VMCPu) after 45min. of antipsychotic drug administration. Data represent mean % control ± SEM (n=8) analysed using one way ANOVA followed by LSD Post hoc analysis *P<0.05 compared to respective vehicle. # P< 0.05 compared to haloperidol.

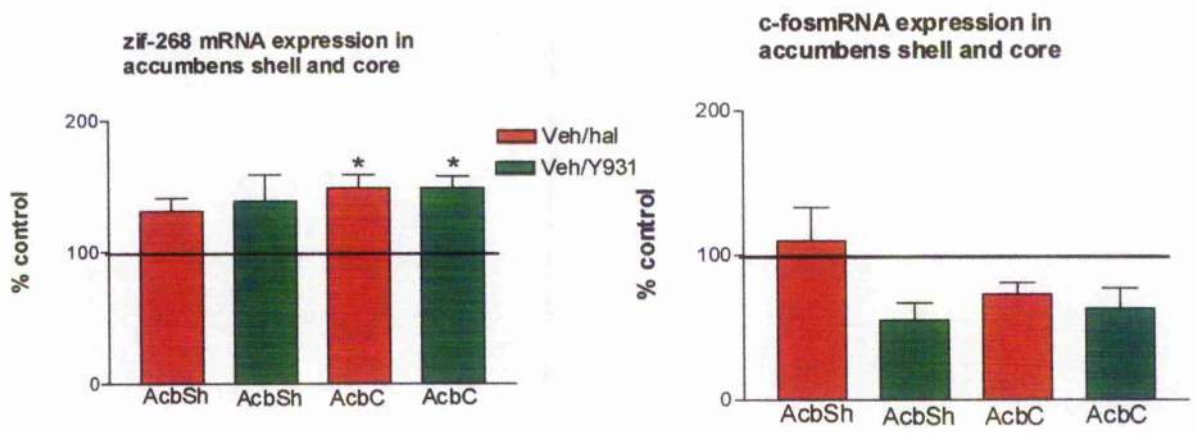


Fig.7. Effect of acute treatment of haloperidol (1mg/kg i.p.) and Y931 (10 mg/kg i.p.), on zif268 and c-fos mRNA expression in the accumbens shell (AcbSh) and core (AcbC), after 45min. of antipsychotic drug administration. Data represent mean % control ± SEM (n=8) analysed using one way ANOVA followed by LSD Post hoc analysis # $P< 0.05$ compared to haloperidol.

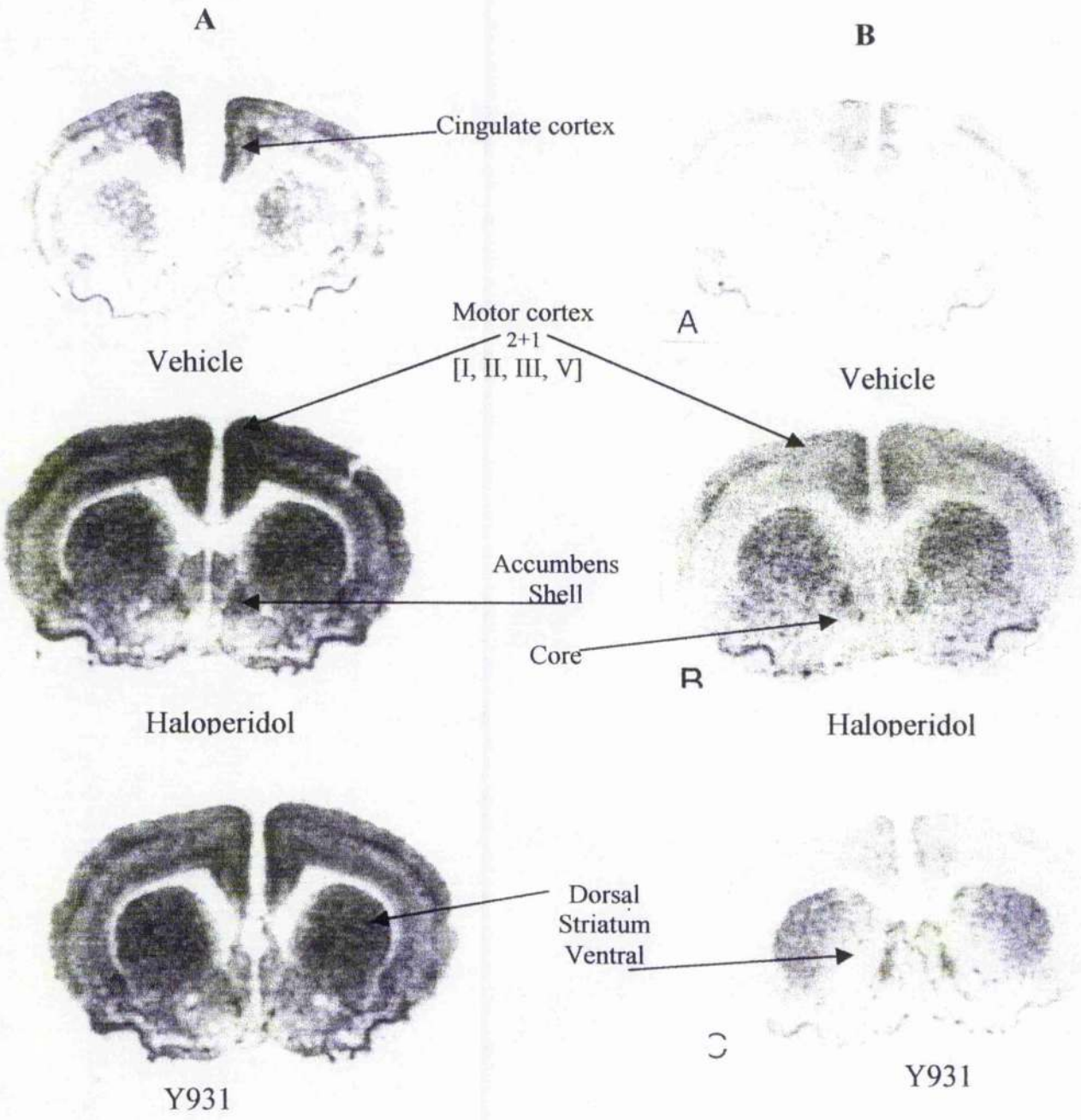


Fig.8. In situ hybridisation autoradiographic localization of Zif-268 (A) and c-fos (B) mRNA expression in different areas rat brain at Level-1. Rats were treated with vehicle (0. 5%HPMC), haloperidol (1mg/kg), and Y931 (10 mg/kg). Brains were collected after decapitation and frozen at --$42^0$ after 45 mins APD treatment.

In the next study of IEG expression, zif-268 and c-fos mRNA were tested after administration of haloperidol (1mg / kg), olanzapine (10 mg / kg) and Y931 (10mg/kg) to compare the IEG expression level of Y931 to that of olanzapine. The relevant dose level of olanzapine for the rats were selected based on its clinical dose equivalence to haloperidol. Results were obtained for zif 268 mRNA expression. (Table 10, page-80).

In this study, haloperidol failed to increase zif 268 mRNA expression level in the motor cortex and other brain regions (cingulate cortex and striatum). Similarly, Y931- induced changes in zif-268 mRNA expression levels were less apparent in the caudate putamen and were not significant.

| Brain regions | Vehicle ROD | Haloper ROD | %Vehicle Control | Olanzap. ROD | %Vehicle Control | Y 931 ROD | %Vehicle Control |
|---|---|---|---|---|---|---|---|
| M1L1 | 0.4± 0.035 | 0.345±0.035 | 84.0 ± 8.4 | 0.31±0.024 | 75.5± 5.7* | 0.228±0.29 | 55.6±7.1 * # |
| M1L2 | 0.31±0.017 | 0.27± 0.019 | 86.2 ± 6.1 | 0.236±0.015 | 75.4± 4.6* | 0.20±0.027 | 64.2±8.4* # |
| M1L3 | 0.39±0.021 | 0.36± 0.037 | 92.8 ± 9.4 | 0.29± 0.021 | 74.9± 5.3* | 0.245±0.030 | 62.8±7.8* # |
| M2L1 | 0.46± 0.040 | 0.374±0.018 | 81.3 ± 3.9 | 0.36± 0.027 | 78.4± 5.9* | 0.295±0.030 | 64.0±6.9* |
| M2L2 | 0.37±0.011 | 0.354±0.018 | 96.4±4.9 | 0.254±0.021 | 69.2 ±5.7* # | 0.25±0.028 | 68.8±7.6* # |
| M2L3 | 0.5± 0.028 | 0.464±0.026 | 92.7± 5.3 | 0.383±0.035 | 76.6±7.1* | 0.345±0.040 | 68.9±8.4* # |
| Cg1 | 0.513±0.10 | 0.546±0.022 | 106.5± 4.2 | 0.472±0.035 | 92.0 ± 6.9 | 0.454±0.030 | 88.5± 6.0 |
| Cg2 | 0.48±0.097 | 0.516±0.028 | 107.2±5.9 | 0.483±0.022 | 100.3± 4.5 | 0.39±0.042 | 81.4± 8.6 |
| DLCPu | 0.23± 0.010 | 0.319±0.043 | 138.4±19 | 0.288±0.017 | 124.9±8 | 0.322±0.050 | 139.6±20 |
| VMCPu | 0.271±0.020 | 0.317±0.040 | 117.2±14.6 | 0.247±0.014 | 91.1± 5.1 | 0.244±0.010 | 90.2±5.0# |

Table 10. Effects of acute haloperidol (1mg/kg), olanzapine (10 mg/kg) and Y931 (10 mg/kg) on zif268 mRNA induction in cortical (Motor cortex-1 Layer1, Motor cortex-1 Layer2 and Motor cortex-1 Layer-3; Motor cortex -2 Layer-1, Motor cortex -2 Layer-2 and Motor cortex-2 Layer -3), Cingulate cortex (Layer –1 and Layer-2) and striatum (DLCPu, VMCPu) and nucleus accumbens (AcbC, AcbSh) brain regions. Data shown as mean optical density (ROD)± SEM and as mean percentage of vehicle control=100%, n=8 per group). Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate.# *$p<0.05$ compared to respective vehicle.

To investigate the results obtained in the motor cortex in more detail as the results were very different than our first IEG experiment, the autoradiographs were re-examined with readings being taken with a higher level of anatomical resolution. This did not reveal any haloperidol-induced changes. However both olanzapine and Y931 significantly reduced zif 268 mRNA expression level in most of the motor cortex (Table 11, page: 82)

| Brain region | Vehicle ROD | Haloperi ROD | %Vehicle Control | Olanzap ROD | %Vehicle Control | Y931 ROD | %Vehicle Control |
|---|---|---|---|---|---|---|---|
| M1L1 | 0.39±0.020 | 0.34±0.034 | 85.9 ± 6.7 | 0.33 ± 0.040 | 84.5 ± 9.1 | 0.26± 0.030 | 66.4± 7.2*# |
| M1L2 | 0.46±0.010 | 0.41±0.028 | 89.8 ± 6.1 | 0.374± 0.040 | 81.6 ± 8.3 | 0.31±0.030 | 68.6± 7.2*# |
| M1L3 | 0.321±0.10 | 0.29±0.019 | 89.7 ± 5.8 | 0.25± 0.020 | 78.1 ±5.3* | 0.24±0.020 | 76.4± 7.3* |
| M1L5 | 0.40±0.020 | 0.36±0.025 | 89.0 ± 6.2 | 0.30±0.014 | 75.4 ±3.5* | 0.29± 0.030 | 71.3± 6.9*# |
| M2L1 | 0.53±0.010 | 0.493±0.031 | 92.9 ± 5.9 | 0.45± 0.034 | 84.7 ± 6.4 | 0.4±0.030 | 74.4± 6.2*# |
| M2L2 | 0.56±0.010 | 0.5±0.025 | 93.3 ± 4.9 | 0.445± 0.050 | 79.7±8.2 | 0.45± 0.040 | 80.5± 7.2 |
| M2L3 | 0.40±0.020 | 0.36±0.025 | 89.0 ± 6.2 | 0.30± 0.014 | 75.4± 3.5*# | 0.29± 0.030 | 71.3± 6.9*# |
| M2L5 | 0.49±0.030 | 0.47±0.025 | 96.3 ± 5.0 | 0.41± 0.020 | 96.3± 3.0* | 0.40± 0.040 | 82.0 ±7.9* |
| M1W | 0.35±0.010 | 0.31±0.017 | 90.1 ± 4.9 | 0.26± 0.020 | 76.7± 2.0* | 0.26±0.020 | 74.1 ±6.1*# |
| M2W | 0.49±0.010 | 0.45± 0.030 | 92.8 ± 6.1 | 0.39± 0.030 | 80.2± 2.9* | 0.38± 0.030 | 78.4± 3.3 |

Table 11. Re-reading of effects of acute haloperidol (1 mg/kg), olanzapine (10 mg/kg) and Y931 (10 mg/kg) on zif268 mRNA induction in cortical (Motor cortex-1 Layer1, Motor cortex-1 Layer2, Motor cortex-1 Layer-3, Motor cortex-1 Layer-5 and Motor cortex as a whole [M1W]. Motor cortex -2 Layer-1, Motor cortex-2 Layer-2 and Motor cortex-2 Layer–3, Motor cortex-2 Layer –5 and Motor cortex as a whole [M2W]. Data shown as mean optical density (ROD)± SEM and as mean percentage of vehicle control=100%, n=8 per group). Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate. *p<0.05 compared to respective vehicle.

A similar pattern of changes was detected with c-fos as with zif268 in this study, as shown in the next Table 12 (page-84). Thus haloperidol produced less marked changes on c-fos mRNA expression than in the previous study, although there were still significant increases on expression level in the caudate putamen. Olanzapine and Y931 also induced significantly higher c-fos mRNA expression in this region, except VMCPu, where it was not significant with olanzapine.

| Brain region | Vehicle ROD | Haloperi ROD | %Control | Olanzap ROD | %Control | Y931 ROD | %Control |
|---|---|---|---|---|---|---|---|
| Cg1 | 0.123±0.020 | 0.120±0.020 | 89.6±14.0 | 0.12±0.010 | 95.4±5.0 | 0.10±0.005 | 81.0±4.4 |
| Cg2 | 0.117±0.020 | 0.120±0.020 | 93.4±15.6 | 0.11±0.010 | 96.7± 9.6 | 0.08± 0.011 | 70.8± 9.6 |
| M1L1 | 0.477±0.010 | 0.035±0.003 | 73.6±6.5 | 0.045±0.010 | 93.5± 23.2 | 0.027±0.004 | 56.2± 7.8 |
| M1L2 | 0.06± 0.015 | 0.042± 0.005 | 73.6±9.1 | 0.052±0.010 | 90.9±20.8 | 0.035± 0.005 | 61.1± 8.2 |
| M1L3 | 0.045± 0.014 | 0.032± 0.007 | 70.6± 14.7 | 0.041± 0.010 | 89.2±21.5 | 0.031±0.006 | 67.6±12.2 |
| M1L5 | 0.046± 0.010 | 0.030± 0.006 | 66.7±13.0 | 0.035±0.010 | 75.2±17.8 | 0.025± 0.004 | 53.9± 8.0 |
| M1W | 0.046± 0.010 | 0.029±0.005 | 62.5± 11.5 | 0.035± 0.010 | 76.2±19.7 | 0.026± 0.004 | 56.4± 7.6 |
| M2L1 | 0.102± 0.020 | 0.069± 0.010 | 67.5± 11.6 | 0.087± 0.010 | 85.6± 11.6 | 0.06± 0.003 | 59.2± 3.2 |
| M2L2 | 0.088±0.018 | 0.062± 0.008 | 70.8± 9.4 | 0.068± 0.013 | 77.2± 15.0 | 0.059± 0.008 | 67.6± 9.2 |
| M2L3 | 0.085± 0.018 | 0.057± 0.009 | 67.6± 11.3 | 0.062± 0.012 | 73.2±13.9 | 0.052± 0.006 | 61.3± 7.0 |
| M2L5 | 0.086± 0.016 | 0.072± 0.012 | 83.2± 14.0 | 0.075± 0.009 | 86.7±10.2 | 0.056± 0.001 | 65.0± 1.5 |
| M2W | 0.090± 0.016 | 0.075± 0.011 | 82.4±13.0 | 0.073± 0.010 | 81.0± 11.6 | 0.060± 0.001 | 65.8± 1.6 |
| DLCPu | 0.012±0.003 | 0.146± 0.053 | 1262±4567* | 0.091± 0.020 | 783±171* | 0.183± 0.020 | 1582± 177* |
| VMCPu | 0.026± 0.006 | 0.082±0.028 | 310± 106* | 0.037± 0.003 | 140± 10..2# | 0.079± 0.008 | 297±29.2* |

Table 12. Effects of acute haloperidol (1mg / kg), olanzapine (10 mg / kg, Y931 (10 mg / kg ) on c-fos mRNA induction (45 mins.after APD) in cortical ( Motor cortex – 1 Layer 1, Layer 2, Layer 3, Layer 5 and whole area[M1W]; Motor cortex–2 Layer -1, Layer-2, Layer-3, Layer-5, Layer-5 and whole area[M2W] ; Cingulate cortex (Layer-1, Layer-2) and striatum ( DLCPu, VMCPu) brain regions. Data shown as mean optical density (ROD) ± SEM and as mean percentage of vehicle control = 100 %, n = 8 per group. Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate. *p<0.05 compared to respective vehicle.

## 2.4.2. Results of in situ hybridisation for marker genes of motor circuits after acute treatment with antipsychotic drugs.

## Effects of haloperidol and Y931 on GAD67 mRNA expression level.

The expression level of GAD67 mRNA 45 minutes after acute treatment with haloperidol and Y931 is shown in the Table 13. The two parts of the substantia nigra regions were investigated, the areas where the GAD 67 mRNA was expressed. The expression levels were not significantly affected after treatment with haloperidol or Y931in comparison to vehicle controls in any of the areas of interest.

| Brain region | Vehicle ROD | Haloperidol ROD | %Vehicle Control | Y931 ROD | %Vehicle Control |
|---|---|---|---|---|---|
| SNL | 0.115±0.009 | 0.122±0.008 | 106.5±6.9 | 0.126±0.014 | 110±12.2 |
| SNR | 0.053±0.009 | 0.62±0.009 | 117.2±16.7 | 0.063±0.007 | 117.2±13.1 |

Table 13. Effect of acute haloperidol (1mg/kg) and Y931 (10 mg/Kg) on GAD67 mRNA expression in substantia nigra lateral (SNL) and substantia nigra reticulata (SNR), 45 mins.after APD treatment. Data shown as mean optical density (ROD) ± SEM and as mean percentage of vehicle control (control = 100 %, n = 8 per group). Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate.

## Effect of haloperidol and Y931 on glucose regulating protein75 (grp75) expression levels after acute treatment.

Effect of acute treatment of haloperidol and Y931 on glucose regulating protein-75 (grp75) is shown in the Table 14. The two areas of the rat brain that expressed substantial number of grp75 containing nuclei were the substantia nigra pars compacta (SNCD) and the subthalamic nucleus (STN). However, the mRNA expression levels were not significantly affected by haloperidol or Y931 treatment when compared to the vehicle controls.

| Brain region | Vehicle ROD | Haloperidol ROD | %Vehicle Control | Y931 ROD | %Vehicle Control |
|---|---|---|---|---|---|
| SNCD | 0.095±0.003 | 0.104±0.003 | 110.1±3.7 | 0.092±0.005 | 97.7±6.0 |
| STN | 0.098±0.016 | 0.102±0.007 | 103.4±7.3 | 0.097±0.007 | 99.0±6.8 |

Table 14. Effect of Haloperidol (1mg/kg) and Y931 (10 mg/kg) on grp75 mRNA expression in the substantia nigra compacta (SNCD) and subthalamic nucleus (STN), 45 mins. after APD treatment. Data shown as mean optical density (ROD) ± SEM and mean percentage of vehicle control (control = 100, n = 8 per group). Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate.

## Effect of haloperidol and Y931 on NOS expression level after acute treatment.

In this experiment the levels of NOS mRNA were measured only in subthalamic nucleus (STN) of rat the brain. However the expression level of NOS mRNAwas not changed by haloperidol or Y931 in this nucleus (Table 15).

| Brain region | Vehicle ROD | Haloperidol ROD | % Vehicle control | Y931 ROD | % Vehicle control |
|---|---|---|---|---|---|
| STN | 0.1716 ±0.017 | 0.166±0.012 | 96.68±6.8 | 0.173±0.0005 | 100.9±0.32 |

Table 15. Effect of acute haloperidol (1mg/kg) and Y931 (10 mg/kg) on NOS mRNA induction in the subthalamic nucleus (STN), 45 mins. after APD treatment. Data shown as mean optical density (ROD) ± and as mean percentage of vehicle control (control = 100%, n = 8 per group). Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate.

### 2.4.3. Summary of the results of the patterns of gene expression induced by acute treatment of Y931 and haloperidol, after 45 minutes of antipsychotic drug treatment.

In the first experiment, treatment with haloperidol (1mg/kg) and Y931 (10 mg/kg), produced wide spread changes in zif268 and c-fos mRNA expression. In contrast, Y931 produced a more restricted pattern of change in IEG expression level.

Haloperidol induced zif-268 mRNA expression significantly in the cingulate cortex, motor cortex M1L2, striatum and nucleus accumbens core but there was no significant change in the accumbens shell and motor cortex M1L1 and M2. Most marked increases occurred in the dorsolateral and ventrolateral caudate putamen, where increases in zif 268 mRNA levels after haloperidol were to 196% and 172% of controls respectively, compared to vehicle controls.

In contrast Y931 produced more restricted pattern of IEG expression. After treatment with Y931, zif268 mRNA expression levels were confined to the caudate putamen (both dorso-lateral and ventro-lateral) and the nucleus accumbens core. These changes were of a similar in magnitude to those produced by haloperidol. Y931 did not cause an increase of zif 268 mRNA expression in the motor cortex that was observed after treatment with haloperidol.

The acute administration of haloperidol produced a substantial increase in the c-fos mRNA expression in all regions of the rat brain, with exception of motor cortex M1 and the accumbens shell and core. The highest increases in c-fos mRNA expression were in caudate putamen, both ventral and dorsal region. In contrast to zif 268 mRNA expression level, the accumbens shell and core did not show significant increases in c-fos mRNA expression level.

The most significant increase of c-fos mRNA expression occurred following the acute treatment of Y931 was in the dorsolateral caudate putamen. In contrast to the zif268 mRNA expression level, the ventral region of caudate putamen did not show a significant

increase in c-fos mRNA expression by Y931. However, there were a similar percentage of increases (≅60%) in both cases. In contrast to zif 268 mRNA expression, c-fos mRNA was not significantly expressed after treatment with Y931in the accumbens core. And after treatment with Y931, c-fos expression level was considerably lower than haloperidol in the accumbens shell.

The most marked increases in the zif268 and c-fos mRNA levels occurred in the dorsolateral caudate putamen after haloperidol treatment was196% and 430% of controls respectively. But after treatment with Y931 the expression level of zif 268 and c-fos mRNA in this area was 231% and 670% of control respectively.

In the second experiment of IEG expression after treatment with haloperidol (1mg/kg), olanzapine (10mg/kg) and Y931 (10mg/kg as before), the altered expression level of zif 268 and c-fos mRNA was not the same in motor cortex as in the first experiment. The expression level of zif268 was significantly lower in all motor cortex areas after treatment with Y931 and olanzapine. The expression level of zif 268 and c-fos mRNA appeared to be lower with Y931 than the olanzapine, but was not significant.

One of the reason for repeating the experiment was to determine the reproducibility of the potentially interesting finding that haloperidol, but not Y931 induce increased IEG expression in most of the motor cortex. For this purpose, we used a more refined image analysis protocol to study IEG expression in individual define layers of the motor cortex. The results from the two studies were still different and we cannot find any obvious reason for this. However, in other areas (caudate putamen, dorsal and ventral) the results were broadly similar in the two studies (but smaller increases), for both zif268 and c-fos mRNA expression.

Haloperidol increased the expression of zif268 and c-fos mRNA dramatically, as expected. This is the first report that showed that atypical antipsychotic drug olanzapine also increases c-fos in the striatum. The increase in expression level of zif268 was not significant in these areas in this experiment. But the overall results are consistent with the

concept that striatal c-fos and zif268 are regulated in parallel. Treatment with Y931 produced a greater induction of striatal c-fos and zif268 mRNA expression level than treatment with haloperidol. This may reflect the high affinity of Y931for $D_2$ receptors.

The other interesting point was that the treatment with Y931 produced more marked increases in c-fos mRNA expression in the dorsolateral caudate putamen. This effect was greater than that which occurred after treatment with haloperidol (670% of control with Y931; 430% of control with haloperidol in experiment-1).

Thus the result of the IEG induction indicates that Y931 modulates striadopallidal neurons with its neurochemical activity.

This is also indicative that Y931 should cause some EPS.

Neither haloperidol nor Y931 altered the expression level of GAD 67, grp-75 or NOS mRNA levels in the discrete region of the basal ganglia after 45mins antipsychotic drug treatment. It was expected that, at least after haloperidol treatment, activity of these nuclei would be altered. Hence these genes are probably not good markers of regional activity, at least, at the time point studied here.

# Chapter 3: Effect of serotonin antagonists, 5-$HT_{2C/2B}$ and 5-$HT_{2C}$ on haloperidol induced catalepsy.

## 3.1. Introduction and Aim.

In the current model of basal ganglia circuitry, blockade of dopamine receptors can result in increased activity of neurons that project from striatum to the external globus pallidus. This may lead to dis-inhibition of the subthalamic nucleus resulting in the increased excitation of the circuits to the internal pallidum. This may cause increased inhibition of thalamo-cortical neurons. Normally there is a balance between dopaminergic and cholinergic tone in the striatum. When this balance is disrupted causing dopaminergic hyperactivity and thalamo-cortical inhibition, Parkinson like symptoms [Extrapyramidal Side Effects (EPS)] may be caused (DeLong , 1990).

Antipsychotics (i.e. the so- called major tranquilisers) are used in the treatment of schizophrenia. In 1950s these agents were adopted for the treatment of schizophrenia because of their selective antipsychotic efficacy and rapidly replaced the previously used medications that were merely sedating. A number of studies compared the effects of these first generation antipsychotic drugs with those of general sedatives such as barbiturates. These studies had shown that these compounds have a specific antipsychotic action that are not possessed by general sedatives (Klein and Davis, 1969). Typical antipsychotic drugs such as haloperidol result in catalepsy in experimental animals, which is an indication of the drug's EPS liability. A similar effect is also expressed, although to a lesser degree, by the atypical antipsychotic drugs, except clozapine. Ddifferent brain regions mediate catalepsy and DA agonist-induced locomotor hyperactivity and stereotypy. The potency of atypical antipsychotic drugs when compared to the potencies of the typical antipsychotics to influence these behaviors may indicate an anatomic specificity of action that confers a favorable spectrum of activity upon the atypical drugs (Kinon and Lieberman, 1996).

Antipsychotic drug induced catalepsy is well known to be due to the blockade of dopamine neuro-transmission via $D_2$ receptors, as catalepsy in rodents or EPS in primates / human is presumed to reflect the activity of the nigrostriatal dopamine (DA) pathway

(Calderon et al., 1988; Costall and Naylor, 1974; Sanberg et al., 1980). The atypical antipsychotic drugs have high affinity for serotonin receptors, $5HT_2$ subgroups, as well as dopamine $D_2$ receptors. But the affinity for $D_2$ receptors of atypical drugs is usually less than for the typical antipsychotic drugs. Biochemical studies have shown that there are reciprocal interactions between DA and $5HT_2$ systems within the CNS and the cataleptic effect of antipsychotic drugs depend on the balance between the dopaminergic and serotonergic system (Balsara et al., 1979). There are convincing reports to suggest that serotoninergic activity of atypical antipsychotic drugs reduce neuroleptic–induced catalepsy (Balsara et al., 1979; Meltzer, 1989, 1996; Poyurovsky and Weizman, 1997; Reavill et.al. 1999). Serotonergic neurons are thought to normally inhibit dopaminergic neurotransmission in the CNS, as a reduction of serotonergic activity increases the functional activity of nigrostriatal DA neurons. The striatum receives serotonergic input from the raphe nuclei, as do the dopaminergic cell bodies in the substantia nigra (SN). Alterations in serotonergic neurotransmission are also known to alter DA-mediated motor behaviours. Chewing and biting, licking or grooming with tongue etc., are examples of motor activity associated with 5HT in adult animals. Cataleptogenic effect of neuroleptics apparently depends on the balance between the two systems (Balsara et al., 1979). The serotonin receptors $5HT_{2A}$ and $5HT_{2C}$ have been implicated in hyperactivity and stereotypy, orofacial dyskinesia, etc. in conjunction with dopamine (Neal-Beliveau et al., 1993; Eberle-Wang et al., 1996).

Atypical antipsychotics have high affinity for serotonin receptors, especially the $5HT_{2A}$ and $5HT_{2C}$ receptors. When serotonergic activity is blocked, dopamine release increases and balances out the dopamine blockade effect at presynaptic receptor site, resulting in putatively less or no EPS (Fox et al., 1998). It had been reported that selective $5HT_{2C}$ and $5HT_{2C/2B}$ antagonists significantly reverse haloperidol induced catalepsy, whereas $5HT_{2A}$ and $5HT_{2B}$ receptor respectively did not reverse haloperidol-induced catalepsy (Di Giovanni et al.,1999; Di Matteo et al., 1999),thus suggesting, that the activity of $5HT_{2C}$ receptors is most probably important in protection against extrapyramidal side effects.

Furthermore, there is evidence, indicating 5-$HT_{2C}$ receptor antagonist, SB-206553 when injected into the substantia nigra pars reticulata produces an antiparkinsonian effect. This may indicate that 5-$HT_{2C}$ receptor antagonism may produce a favourable result in the situation of motor disturbance (Eberle-Wang et. al., 1996; Fox et. al., 1998). However, few drugs have differential affinity for $5HT_{2B}$ and $5HT_{2C}$ receptors, and the role of $5HT_{2B}$ and $5HT_{2C}$ receptors in reducing EPS has not been explored in very many studies.

Y931 has high affinity for dopamine $D_2$ receptors, showing high $D_2$ receptor blockade in the striatum. It also has very high affinity for $5HT_2$ receptors, specifically $5HT_{2C}$ receptors. The evidence suggests that Y-931 has very little tendency to produce EPS. Because of this, our aim for this part of the experiment is to look into the effects of $5HT_{2C}$ receptor antagonists on haloperidol induced catalepsy. If the $5HT_{2C}$ receptor antagonists reduce the haloperidol-induced catalepsy and produce a mRNA expression profile like that of Y931, that may explain the method of activity of Y931 in the reduction of EPS.

We have also measured the different gene expression levels of specific marker genes of different areas of basal ganglia on the brain tissues of these rats to map the gene expression three hours after treatment with the drugs. This might help us to find out any change in the mRNA expression level in the presence of $5HT_2$ antagonists after treatment with haloperidol, Y931 and olanzapine. The pharmacological profile of Y931 is very similar to that of the atypical antipsychotic drug, olanzapine. So we have decided to compare the gene expression level of Y931 with this atypical antipsychotic drug as well as the typical drug haloperidol. Comparison of these results would help us to understand the mechanism of activity of Y931 and to find out if Y931 is an atypical drug with lower EPS.

The catalepsy tests were used to check EPS liability of antipsychotic drugs, as these tests have high predictive accuracy (Sanberg et al., 1988; Wadenberg and Hicks, 1999).

Thus two methods were used for this part of the study:

a) Animal behavioral Test (measurement of catalepsy).

b) In situ hybridisation (measurement of site specific gene expression level which could give indication of metabolic activity).

## 3.2. Materials and methods

| Name of the drugs | Source | Dosage |
|---|---|---|
| 1. Haloperidol | RBI | 1 mg / kg |
| 2. Olanzapine | Welfide Corporation | 10 mg / kg |
| 3. Y931 | Welfide Corporation | 10 mg / kg |
| 4 SB 200646 (SB-1) $5HT_{2C/2B}$ | TOCRIS | 10 mg / kg |
| 5. SB 242084 (SB-2) $5HT_{2C}$ | SIGMA - RBI | 10 mg / kg |

Table 16. Drugs used for animal behavioral experiment. Olanzapine (Moor et al., 1992; Beasley et al. 1996) an atypical antipsychotic drugs in use. SB 200646 is a selective 5-$HT_{2C/2B}$-receptor antagonist with $pk_i$ value 6.9 for serotonin $2_C$ receptors and with pi value 7.5 for serotonin $2_B$ receptors. SB200646 has 50-fold selectivity for 5-$HT_{2C}$ and 5-$HT_{2B}$ over 5-$HT_{2A}$ receptors (Kennett et al., 1994). SB 242084, a selective 5-$HT_{2C}$ receptor antagonist with $pk_i$ value 9.0 for serotonin $2_C$ receptors. It has 100 and 158-fold selectivity over the 5-$HT_{2B}$ and 5-$HT_{2A}$ receptors subtypes respectively. Thus SB242084 is a useful tool to evaluate the effect of selective blockade of 5-$HT_{2C}$ receptors. (Matteo et al., 1999). The relevant dose levels of olanzapine for the rats were selected based on its clinical dose equivalence to haloperidol.

Forty male hooded Long Evans rats (225g -290g) were used for this experiment. The rats were housed in-group of 5 in each cage under a 12 hours light and dark cycle with free access to food and water. The animals were allowed one week of adaptation to

laboratory conditions before being used in experiment. The animals were randomly allocated to six groups:

| | |
|---|---|
| Group A. | Vehicle / Vehicle |
| Group B | Vehicle / Haloperidol |
| Group C | SB1. / Haloperidol |
| Group D | SB2 / Haloperidol |
| Group E | Vehicle / Olanzapine |
| Group F | Vehicle / Y931 |

In each case vehicle was 0.5% HPMC as before. Because of the number of animals and several stages of experiment, the study was conducted in two days.

### 3.2.1.Animal behavioural Test:

### Catalepsy Test

The classic tests for dopamine (DA) antagonist activity is to measure the ability of a drug to induce a state of catalepsy in rodents (Costall and Naylor, 1974). Functional responces elicited by DA agonists include activation of locomotor activity, steriotypy and rotation in rats with unilateral 6-oHDA-induced lesions of the nigrostriatal pathway.

Catalepsy is a phenomenon defined as the long -- term endurance of an abnormal posture by the experimental anima after antipsychotic drug treatment l, without being able to correct it. When a normal animal is placed in an unusual posture, it will change its position within seconds. In contrast, cataleptic animals will maintain this posture for

several minutes or longer, dependent upon the degree of catalepsy. The catalepsy tests have been widely used as a preclinical test to predict and motor side-effect liability of antipsychotic drugs, i.e., EPS liability of new antipsychotic drugs. These tests have high predictive accuracy (Sanberg et al., 1988; Wadenberg and Hicks, 1999) to measure EPS. Catalepsy is observed with typical and atypical antipsychotic drug treatment, when doses used produce $D_2$ receptor occupancy ≥85%.

A typical catalepsy test consists of placing an animal into an unusual posture and recording the time taken to correct this position (i.e., the four paws of the experimental rat should be extended and abducted, Morelli and Chiara, 1985). This time is regarded as an index of the intensity or degree of the catalepsy. A variety of apparatus can be used - including wire grids, single bar, parallel bars, platforms or pegs to situate the animals in an unusual position. Whilst catalepsy is a robust behaviour, its absolute intensity may be affected by minor alteration in various experimental and environmental parameters such as repeated testing and handling (Sanberg et al., 1988; Di Chiara et al., 1979; Ögren etal., 1986).

Two methods of catalepsy testing were employed in the present studies. These are described below.

## A. Block Test

The rats were positioned with their forelimbs placed on top of a 10 cm high metal block and the hind limbs on the bench. The length of time the rats maintained this position, without a deliberate move to step down, was recorded by stop - watch. The rats were judged to be cataleptic if they maintained their position for >30 sec. or more on the block without trying to move from the set position. Catalepsy was measured up to a maximum of 2.5 mins. This procedure was conducted both 60 min. and 120 min. following antipsychotic drug administration.

The tests were measured 'Blind' with the person recording the activity unaware of the treatment the rat had received in a 'quiet' room.

180 min. post antipsychotic drug treatment, the rats were sacrificed by decapitation (Kalkman, et.al.1998)

The observed data for time spent on the block were plotted in graph form as means ±SEM after one way ANOVA was carried out.

## B. Grid Test

A vertical (angle, $90^0$) 10 cm. high grid, made with a wire mesh screen, was placed on the bench. The animals were placed on the grid, with all four paws on the wire mesh in a 'frog' posture. The use of such an unnatural posture is essential for the specificity of this test for catalepsy (Morelli and Chiara, 1985). The time rat remained in the same position without moving any of their paws, was then measured for a maximum of one minute. The time at which the animal made the first paw movements were measured. An animal was considered cataleptic, when the animal stayed immobile on the same position for >30 sec.

The tests were measured 'Blind' as before.

All the observed data for the time rats spent on the grid were then plotted as means ±SEM after one way ANOVA was carried out.

Animals were pretreated with either the 5HT2C/2B receptor antagonist SB200646 or the 5HT2C receptor antagonist SB242084 and their ability to modify haloperidol-induced catalepsy was examined. Parallel groups, comprised a Y931 group and an olanzapine group. The experimental protocol is summarised in table 17 (page-100).

| −15 mins. (pretreatment) | T = 0 | +60 mins<br><br>Test - 1 | +120 mins<br><br>Test - 2 | +180mins<br><br>animals sacrificed |
|---|---|---|---|---|
| Pretreatment (i.p.)<br>Vehicle (0.5%HPMC) or SB1 (10mg/kg) or SB2 (10 mg/kg) | Treatment (i.p.)<br>Vehicle (0.5%HPMC) or Haloperidol (1 mg/kg) or Olanzapine (10mg/kg) or Y931 (10 mg/kg) | 1. Block Test<br><br>2. Grid Test | 1. BlockTest<br><br>2. Grid Test | Animals were sacrificed |

Table 17. Outline of the experiment on animal behaviour upon haloperidol-induced catalepsy and effect of SB1 (5 $HT_{2C+2C}$ antagonist) and SB2 (5 $HT_{2C}$ antagonist).

After animals were sacrificed, the brains were collected in the usual manner as before, for in situ hybridisation analysis (4.2.).

## Results of block tests and grid tests:

It is evident from the table 18 and from Fig. 9 and 10 that at 60 minutes post APD, there was no emergence of catalepsy, after the haloperidol treatment. Olanzapine and Y931 both appear cataleptic according to the block test at the later time, but the behaviour of these animals were not entirely consistent with cataleptic behaviour. The sedative effects (most probably due to the blockage of histamine receptors) by olanzapine and Y-931 made it difficult to judge catalepsy in these animals. Similar physical conditions in the experimental animals treated with antipsychotic drug, were also described by other authors (Costall and Naylor, 1975). There was a tendency for SB 200646 to reduce the cataleptic effect of haloperidol, as assessed by the block test, at 120 min, but this was not significant. There was no such effect by any of the $5HT_{2C}$ antagonists with the grid test.

| Treatment | Block Test | | | | Grid Test | | | |
|---|---|---|---|---|---|---|---|---|
| | Test-1 Mean Time (Sec.) | No of animals catalept in each gp. | Test-2 Mean Time (Sec.) | No of animals catalept in each gp. | Test-1 Mean Time (Sec.) | No of animals catalept in each gp. | Test-2 Mean time (Sec.) | No of animals catalept in each gp. |
| Veh / Veh | 1.3±0.29 | 0 / 6 | 7.0±2.0 | 0 / 6 | 3.8±2.7 | 0 / 6 | 12.2±3.8 | 1 / 6 |
| Veh / Hal | 7.5±4.2 | 1 / 8 | 42.0±25.2 | 3 / 8 | 7.6±3.5 | 0 / 8 | 38.5±8.5 | 4 / 8 |
| SB1 / Hal | 6.9±4.3 | 1 / 7 | 16.0±2.8 | 1 / 7 | 10.0±3.0 | 0 / 7 | 27.4±7.0 | 3 / 7 |
| SB2 / Hal | 17.0±7.5 | 1 / 7 | 37.7±8.8 | 5 / 7 | 17.3±6.7 | 1 / 7 | 28.2±8.5 | 2 / 7 |
| Veh/Olanz | 14.6±5.4 | 1 / 6 | 130.8±49 | 3 /6 | 5.5±3.2 | 0 / 6 | 19.7±8.4 | 2 / 6 |
| Veh/Y931 | 27.7±14.7 | 1 / 6 | 48.3±14.7 | 4 / 6 | 5.8±2.4 | 0 / 6 | 14.0±6.8 | 1 / 6 |

Table 18. Effect of treatment on groups of rats with haloperidol (1mg/kg), haloperidol and SB1 (10mg/kg, i.p.), haloperidol and SB2 (10 mg/kg, i.p.), olanzapine (10mg/kg, i.p.) or Y931 (10mg/kg, i.p.) on the cataleptic behaviour of rats. Catalepsy was assessed as amount of time rats spent on the block or grid for >30 sec. Results are expressed as the mean ± SEM after one way ANOVA with each treatment.

## Effects of haloperidol and haloperidol +SB200646 (SB 1) or haloperidol +SB242084 (SB2) in comparison to the effect of olanzapine and Y931

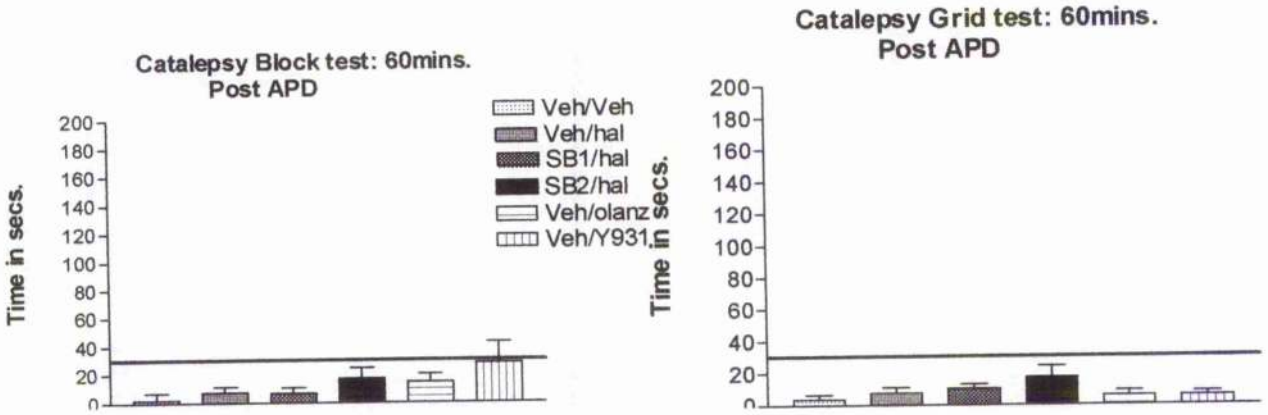


Fig.9. Catalepsy Block Test and Grid Test: Effects of haloperidol (1mg/kg i.p.) and haloperidol +SB200646 (SB 1, 10 mg/kg, i.p.) or haloperidol + SB242084 (SB2, 10 mg/kg, i.p.) in comparison to the effect of olanzapine (10 mg/kg) and Y931 (10 mg/kg) 60 mins after drug treatment.

Catalepsy was assessed as amount of time rats spent on the block or grid for >30 sec. Results are expressed as the mean ± SEM time spent by each group of rats after one way ANOVA with each treatment. The number of animals in each treatment group are 6-8 as shown in Table 18.

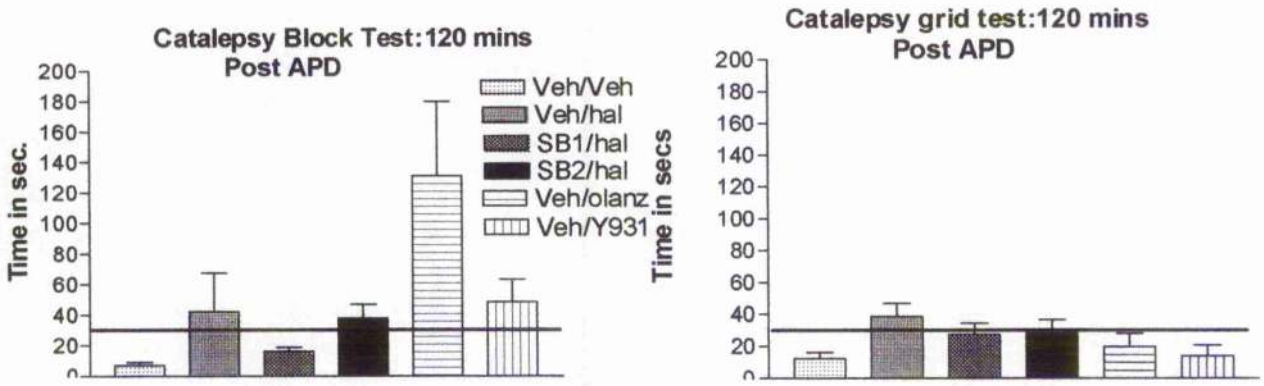


Fig.10. Catalepsy Block Test and Grid Test: Effects of haloperidol (1mg/kg i.p.) and haloperidol +SB200646 (SB 1, 10 mg/kg, i.p.) or haloperidol +SB242084 (SB2, 10 mg/kg, i.p.) in comparison to the effect of olanzapine (10 mg/kg) and Y931 (10 mg/kg) 120 mins after drug treatment.

Catalepsy was assessed as amount of time rats spent on the block or grid for >30 sec. Results are expressed as the mean ± SEM time spent by each group of rats after one way ANOVA with each treatment. The number of animals in each treatment group are 6-8 as shown in Table 18.

## 3.2.3. Modified catalepsy test: Dose related effects of haloperidol on catalepsy, using 'BAR TEST'.

Since the results with the catalepsy tests after haloperidol treatment were not totally clear in the first experiment, it was decided to repeat the experiment using two doses of haloperidol: 1mg/kg i.p. and 2mg/kg i.p.

The method for the animal behaviour test was changed using bar method, the protocol of Reavill et al., 1999 to increase reproducibility.

- Instead of a 10 cm. Block, a 1 cm. diameter metal bar on a retort stand, raised 10 cm above bench level was employed. The front paws were gently placed on the horizontal metal bar. It is important that four paws be well extended.

- The measurement of catalepsy:

- If the rats climbed down from the bar straight away, they were put back on the bar again. This 'training' element was done for a maximum of three times, as it had been reported that catalepsy measures are more reliable if the animals experience the test repeatedly (Reavill et al.1999).

- Tests were conducted at 30mins, 60mins, 90 mins, and 180 mins. The observed data for the time rats spent on the bar at these various time points, were then plotted as means ± SEM after one way ANOVA was carried out.

- The rat was deemed cataleptic if it stayed on the bar for 30 sec or longer. If the rats were cataleptic they were given a score 1, if not they scored 0. If it stayed on for 30 sec., it was kept on the bar for maximum of 2 mins and then taken off the bar, unless it got down by itself.

## Materials and Methods

Drugs and other materials were from the same sources as before and were prepared same way as before.

Twelve male hooded Long Evans rats (225g - 300g) were used for this experiment as before. The rats were housed in two groups under 12hr light and dark cycle with free access to food and water. The animals were allowed one week to adapt to the laboratory conditions before experiment. The animals were randomised and were divided into four groups:

| | | |
|---|---|---|
| A. | Veh = Vehicle (0.5% HPMC) | 1ml / kg |
| A. | Hal.1= Haloperidol | 1mg / kg |
| B. | HAL.2= Haloperidol | 2 mg / kg |
| C. | Y931 = Y931 | 10 mg / kg |

The observed data for time spent on the bar were plotted in graph form as means ±SEM after one way ANOVA was carried out.

## 3.2.4.Results

In this experiment, haloperidol induced clear catalepsy at the 2mg/kg dose at all time points. The emergence of catalepsy in rats treated with 1mg/kg haloperidol was not apparent until three hours after drug treatment. In the two animals that received Y931, catalepsy was again difficult to assess because of the 'sedatory' effects of Y931 (Table-19, fig: 11, page: 105-106).

| Treatment | BAR TEST | | | |
|---|---|---|---|---|
| Vehicle | Test -1 (30mins after APD)mean ± SEM (sec.) | Test – 2 (60 mins after APD) mean±SEM (sec.) | Test – 3 (90 mins. after APD) mean±SEM (sec.) | Test – 4 (180mins. after APD) mean± SEM (sec.) |
| Vehicle n=2 | 1 ± 0.35 | 2.5 ± 1.8 | 6.5 ± 6.5 | 13.5 ± 1.06 |
| Haloperidol 1mg / kg n=4 | 0 ± 0 | 4.24 ± 1.9 | 11.0 ± 2.8 | 79.5± 31.7 |
| Haloperidol 2mg / kg n=4 | 12.8 ± 4.5 | 71.75± 25.0 | 96.25 ±20.6 | 120 ± 0 |
| Y931 (10 mg / kg) n=2 | 0 ± 0 | 13.5±7.4 | 75.0 ± 37.5 | 70.0±35.46 |

Table 19. Table showing the time animals stayed on the bar treatment with 1mg / kg and 2mg / kg , i.p. of haloperidol or 10 ng/kg Y931. Catalepsy was assessed as amount of time rats spent on the bar for >30 sec. Results are expressed as the mean ± SEM time spent by each group of rats after one way ANOVA with each treatment.

**Effect of different dosage of haloperidol on catalepsy at 90 mins. after APD administration**

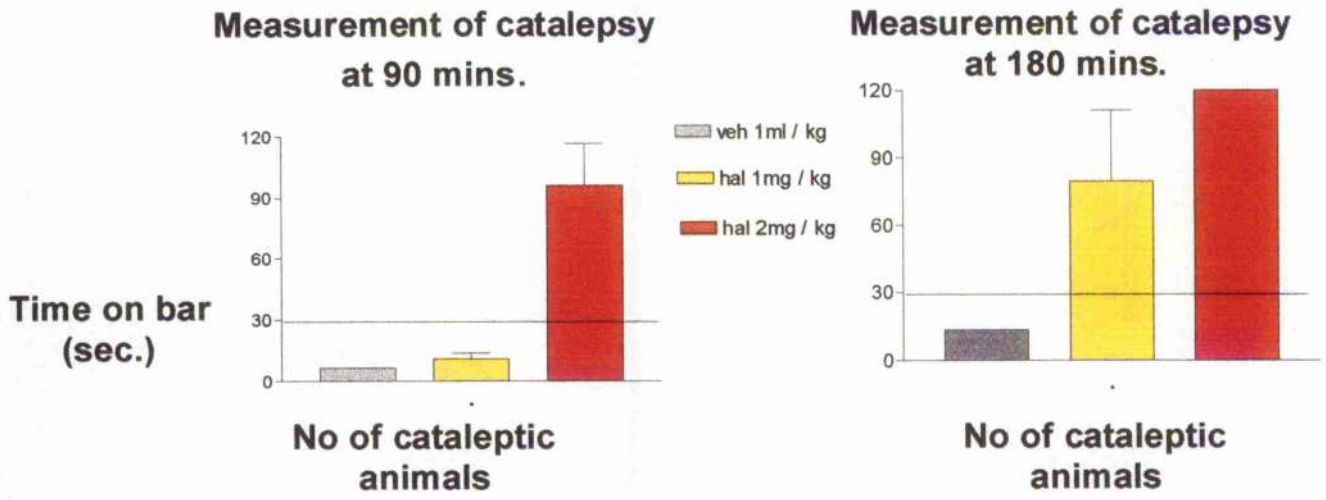


Fig.11. Catalepsy Bar Test: Effects of haloperidol (1mg/kg i.p. and 2 mg/kg i.p.) after 90 mins and 180 mins of drug treatment. Catalepsy was assessed as amount of time rats spent on the bar for >30 sec. Results are expressed as the mean ± SEM time spent by each group of rats after one way ANOVA with each treatment.

### 3.3. Animal behavioural Test (Repeat):

Based upon the results of the previous experiment it was decided to repeat the experiment with serotonin $5HT_{2C/B}$ antagonist SB200646, to investigate its effect on haloperidol-induced catalepsy using haloperidol at a dose of 2 mg/kg.

## Materials and methods:

### Drugs used:

| | |
|---|---|
| 1. Haloperidol | 2mg /kg |
| 2. Olanzapine | 10 mg / kg |
| 3. 5HT2c/$2_B$ | 10 mg / kg |
| (SB 200646 = SB1) | |

Sources of the drugs were as before.
Drugs were prepared as described previously.

Twenty-four male hooded Long Evans rats with a weight range of . 225 – 300g were used for this experiment. The animals were randomly allocated to four treatment groups.

A. Haloperidol

B. Olanzapine

C. SB1+ haloperidol

D SB1 + olanzapine

Six animals were allocated in each group.

| 0 Time – 1 | Time-2 + 5 mins. | 30mins. APD | 60 mins. APD | 90mins. APD | 180 mins. APD |
|---|---|---|---|---|---|
| Treatment 1 (i.p.) Vehicle (0.5%HPMC) or SB1 (10 mg/kg) | Treatment 2 (i.p.) Haloperidol (2 mg/Kg) or Olanzapine (10 mg/kg) | Test -1 | Test -2 | Test -3 | Test -4 |

Table 20. Outline of the experiment, the effect of $5HT_{2B/C}$ antagonist on haloperidol-induced catalepsy.

## 3.3.1.Results

In this experiment haloperidol (2mg/kg) produced clear catalepsy which was present in all animals 90 min post drug treatment. Whilst animals pretreated with the 5HT2B/C receptor antagonist SB 200646 appeared to show reduced catalepsy. This was not significant. Olanzapine appeared to produce catalepsy in about 50% of animals three hours post drug treatment, whereas in the group receiving SB1 pretreatment, all rats displayed catalepsy (Table 21 and Fig-12, page:109-110).

The observed data for time spent on the bar were plotted in graph form as means ±SEM after one way ANOVA was carried out.

| Treatment | BAR TEST | | | |
|---|---|---|---|---|
| | Test- 1<br>(30 mins after APD)<br>mean ±SEM<br>(sec.) | Test- 2<br>(60 mins after APD)<br>mean ±SEM<br>(sec.) | Test- 3<br>(90 mins after APD)<br>mean ±SEM<br>(sec.) | Test- 4<br>(180 mins after APD)<br>mean ±SEM<br>(sec.) |
| Veh + hal<br>n=6 | 8.5 ± 2.0 | 69.2 ± 20.8 | 108.3 ± 9.0 | 125.5 ± 1.8 |
| SB1+hal<br>n=6 | 12.8 ± 9.5 | 37.2 ± 17.7 | 87.5 ± 8.4 | 101.7 ± 4.7 |
| Veh+ Olan<br>n=6 | 1.5 ± 0.8 | 6.8 ± 2.4 | 30.3 ± 8.6 | 54.5 ± 7.9 |
| SB1+ Olan<br>n=6 | 7.2 ± 3.2 | 30.5 ± 18.5 | 24.5 ± 3.7 | 57.8 ± 4.3 |

Table 21. Table showing the time animals stayed on the bar after pretreatment with SB1 (10 mg/kg, i.p.) + haloperidol (2 mg/kg, i.p.) or olanzapine (10 mg/ kg). SB1 was administered 5 mins. before haloperidol or olanzapine. Numbers of animals were six in each group. Catalepsy was then measured at 30, 60, 90 and 180 mins. post drug administration using the bar test as described before. Catalepsy was assessed as amount of time spent on on bar. Catalepsy was defined if the animals stayed >30 sec. on bar. After the 180 mins rats were killed by decapitation. Results are expressed as the mean ± SEM time spent on the bar.

Method was as per Reavill et. al., 1999.

**Influence of SB200646, a $5HT_{2C/B}$ receptor antagonist on haloperidol induced catalepsy after 90 mins. APD**

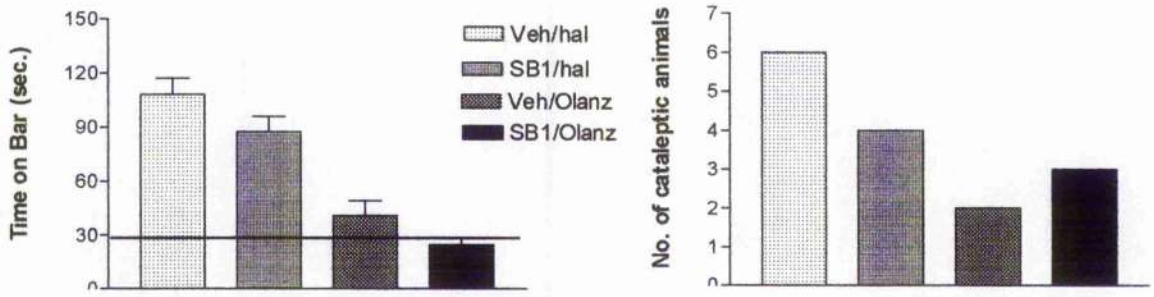


Fig.12. Catalepsy Bar Test: Effects of haloperidol (2mg/kg i.p. and with pre-treatment of SB1, 10mg/kg i.p) and olanzapine (10 mg/kg i.p. with pre-treatment of SB1, 10 mg/kg, i.p.) after 90 mins of drug treatment. SB1 was administered 5 mins. before haloperidol or olanzapine. Number of animals were six in each group.

Catalepsy was defined if the animals stayed >30 sec. on bar. After 180 mins rats were killed by decapitation. Results are expressed as the mean ± SEM time spent on the bar after one way ANOVA with each treatment.

## 3.4. Summary of results for animal behavioral experiments

The catalepsy tests are used to check EPS liability, as it is believed that these tests have high predictive accuracy for motor function (Wadenberg and Hicks, 1999)

The 1mg / kg haloperidol dose did not produce reproducible catalepsy in the animals. Rats treated with olanzapine and Y931 appeared cataleptic. But the behaviour of these animals was not entirely consistent with the cataleptic behaviour. Both olanzapine and Y931 have high affinity for Histamine (H1) receptors, which could cause sedation, that would make the animal's movement sluggish and may make them stay on the block or grid or bar longer. This could be misread as catalepsy. Since the behaviour of these animals appeared sluggish rather than incapable of movement, these results should be interpreted with caution.

Serotonin receptor antagonists, specifically the 5HT2 (specifically $5HT_{2B+2C}$ / $5HT_{2C}$) receptor antagonists have been proposed to attenuate haloperidol induced catalepsy (Meltzer et al., 1995., 1996; Wadenberg and Hicks, 1999; Reavill et al., 1999).

In the combined drug treatment, although 1mg / kg haloperidol dose did not produce catalepsy in a significant number of animals, nevertheless, there was a tendency for SB 200646 (SB1) to reduce the cataleptic effect of haloperidol, as assessed by the block test at a later time point (120 mins.). However this was not a significant reduction.

The 2 mg / kg haloperidol dose effectively produced catalepsy in all the animals in the group at 90 mins. onwards. Thus confirming the fact acknowledged by other researchers (Kapur et al., 1999, 2000a) that catalepsy or extrapyramidal side effect (EPS) of haloperidol and other typical antipsychotic drugs is dose dependent. Our results show that, in acute treatment, the dose of haloperidol needed to produce catalepsy would be more than 1mg/kg.

In second experiment, pretreatment with the $5HT_{2B+C}$ antagonist SB 200646, did not significantly attenuate haloperidol induced catalepsy. But the result of combined treatment of SB1+ olanzapine was not as it was expected. Again this could be because of the sedative effect of histamine receptor antagonism.

Thus the result of the catalepsy test concluded that the addition of drug SB200646, a $5HT_{2C/2B}$ receptor antagonist has minimal influence on the properties of haloperidol to induce catalepsy. These results do not support a clear role for $5HT_{2(C+2B}$ receptors in protection against extrapyramidal side effect.

There was not enough time for in situ analysis study on the brain tissues from this study which may had given us further information at the mRNA expression level.

# Chapter 4: Comparison of motor circuit marker gene mRNA expression levels after treatment with haloperidol olanzapine and Y931: Effect of $5HT_2$ antagonists on haloperidol-induced changes in gene expression

# 4.1.Introduction and Aim

According to one of our hypotheses, Y931 acts outside the striatum to subdue the activation of the indirect pathway via its significantly high affinity for $5HT_{2C}$ receptors. Y931 may act to suppress EPS at a site downstream of the striatum. $5HT_{2C}$ receptors antagonist property of Y931 may be involved in reducing EPS.

We have pretreated the animals with $5HT_{2C}$ receptors antagonist 5-HT $_{2C+2B}$ and 5-$HT_{2C}$ before haloperidol treatment. The pretreatment with these serotonin antagonists may change result of haloperidol treatment alone and may reflect on the mRNA expression level. If this result shows that the mRNA expression level of marker genes at site or sites downstream of striatum is similar to that of Y931or olanzapine, that will suggest that $5HT_{2C}$ is involved in the activity of Y931.

There are reports that the expression of certain enzymes and proteins that are involved in neuromodulation are regulated in response to altered activity in the indirect pathway. These neurochemical components could be used as markers for different regions of basal ganglia activity and their influence by neuroleptic drugs. Some of these molecules are: GAD 67, the enzyme responsible for keeping GABA levels at steady state, which are regulated in response to the altered state of the indirect pathway; nitric oxide synthase (NOS) which is known to be involved in the neuromodulation in STN; glucose regulating protein (grp75), a protein related to hsp70, which is regulated in response to altered activity in the indirect pathway in STN; and parvalbumin, a calcium binding-neuroprotective protein involved in neuromodulation, a useful marker for GABAergic neuronal subtype (Hontanilla et al., 1997). Thus monitoring the levels of these markers in addition to IEGs will help us to establish some characteristics of Y931 that differ from

haloperidol and olanzapine in its effect on the direct and indirect pathways of the basal ganglia.

In this part of the study, changes in gene expression were monitored 3 hours after drug administration. Genes such as GAD 67, NOS and grp 75 did not show any change in the mRNA expression levels compared to the controls after 45 mins (see Table 13,14 and 15 in chapter 2, section 2.4.2.). Since these genes are not IEGs, there was a possibility that they could be affected by antipsychotic drugs treatment at a later time point.

Because fra-2 is induced more slowly than other IEGs such as c-fos and zif268, fra-2 mRNA expression levels were also measured at this time point, in this study.

## 4.2. In situ hybridisation

### 4.2.1. Materials and method

All the materials for this part of the experiment are as in the animal behaviour experiment in the section 3.2 (page-95). The brain tissue from the animal behaviour experiment in section 3.2.1(page-96) was used for this part of experiment in chapter. The in situ hybridisation was conducted as previously described for in situ hybridisation of IEGs mRNA expression (see Chapter 2, sections - 2.3, 2.3.1 and 2.3.2, pages-61-65), unless it is mentioned otherwise.

### Oligonucleotide probes:

Oligonucleotide probe for fra-2 (45 mer) complementary to bases 900-945 of human fra-2 mRNA sequence (Kamme et al 1995).

fra-2 (45 mer) 5′ TAG TTG TTC CCC GCT GCT ACT GCT TCT GCG GTG AGC CTT GGA GCA 3′

The oligonucleotide probe for parvalbumin was also from GenBank:

Parvalbumin (45 mer) 5′ CCC CAG CTC ATC CTC CTC AAT GAA GCC ACT TTT GTC TTT GTC CAG 3′

## 4.2.2. Results

### Effect of haloperidol, haloperidol+5-$HT_2$ antagonists, olanzapine and Y-931 on the Fra-2 mRNA expression levels.

The results of fra-2 expression levels are shown in the table 22 and Fig.13. The results showed that there was no change in fra-2 mRNA expression level in any of the regions investigated after treatment with any of the three drugs used. However, in the dorsal caudate putamen the effect of treatment approached overall significance ($P<0.077$) producing higher fra-2 mRNA expression levels in comparison to the controls.

In general, the fra-2 mRNA expression level in presence of Y931 was very similar to that of haloperidol in all areas of motor cortex, cingulate cortex and caudate putamen, although these changes did not reach significance. Neither of the $5HT_2$ antagonists were able to change the haloperidol induced changes in any of the areas (Table 22, fig: 13, page 118-119).

| Brain region | Veh/Veh | Veh/Hal | | SB1/Hal | | SB2/Hal | | Olanz | | Y931 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ROD | ROD | %Con | ROD | %Con | ROD | %Con | ROD | %Con | ROD | %Co |
| M1L1 | .122±0.013 | .113±.014 | 92.9±11.4 | .114±.005 | 93.43.6 | .096±.008 | 79.2±6.3 | .098±.010 | 80.6±7.8 | .110±.009 | 90.2±7.4 |
| M1L2 | .055±.007 | .052±.006 | 94.6±10.9 | .053±.004 | 95.1±7.0 | .053±.005 | 96.0±9.8 | .051±.010 | 91.5±12.5 | .055±.009 | 99.6±1.7 |
| M2L1 | .127±.013 | .120±.010 | 94.2±10.3 | .112±.010 | 88.1±6.4 | .114±.008 | 89.5±6.6 | .105±.012 | 82.2±9.0 | .126±.011 | 99.0±8.4 |
| M2L2 | .088±.007 | .091±.010 | 103.3±9.6 | .083±.010 | 94.9±10. | .077±.007 | 88.5±8.0 | .069±.009 | 78.9±10.7 | .085±.012 | 97.3±14 |
| Cg1 | .130±.012 | .118±.010 | 91.1±6.0 | .115±.010 | 88.8±7.2 | .126±.007 | 96.9±5.1 | .097±.008 | 75.0±6.2 | .107±.019 | 82.5±14 |
| Cg2 | .130±.012 | .118±.010 | 91.1±6.0 | .115±.010 | 88.8±7.2 | .126±.007 | 96.9±5.1 | .097±.008 | 75.0±6.2 | .107±.019 | 82.5±14 |
| DLCPu | .072±0.01 | 0.13±.020 | 183.0±30 | .137±.020 | 190±29 | .141±.018 | 195±24.6 | .125±.014 | 173±19.5 | .153±.012 | 212±16 |
| VMCPu | .091±.011 | .082±.010 | 90.6±6.3 | .080±.010 | 88.4±5.2 | .080±.005 | 93.5±10.4 | .061±.008 | 67.3±9.1 | .074±.005 | 81.7±5.2 |

Table 22. Results of in situ hybridisation showing the effects of haloperidol (1mg/kg) and haloperidol with SB1 (10mg/kg) or SB2 (10mg/kg) on fra-2 mRNA expression three hours after APD, in comparison to the effect of olanzapine (10 mg/Kg) or Y93 (10 mg/kg) in cortical (M1 Layer-1and Layer-2; M2 Layer-1 and Layer-2), Cingulate cortex (Cg Layer-1, Layer-2) and striatum (DLCPu,VMCPu). Data shown as mean optical density (ROD) ±SEM and mean percentage of vehicle control (control=100%, n=6-8 per group). Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate.

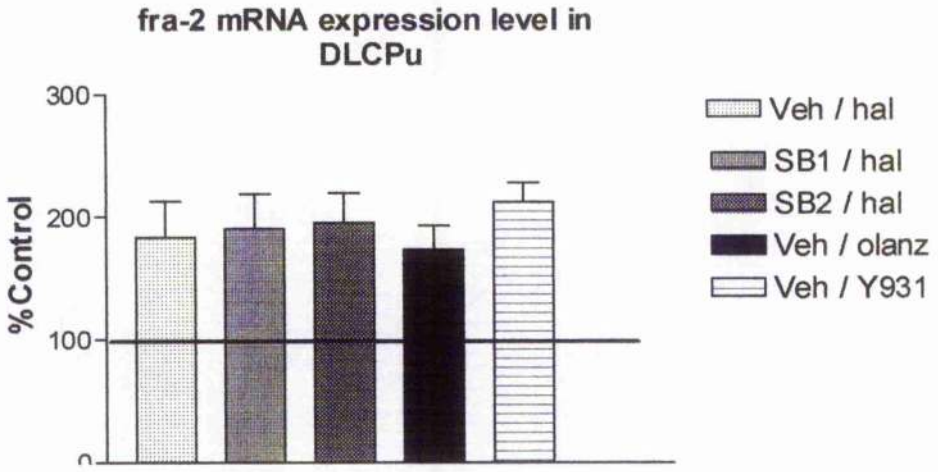


Fig 13. Effect of haloperidol, haloperidol+$5HT_2$ receptor antagonists, olanzapine and Y-931 on fra-2 mRNA expression levels in dorsolateral caudate putamen (DLCPu), three hours after APD treatment.

Data shown as mean optical density (ROD) ±SEM and mean percentage of vehicle control (control=100%, n=6-8 per group). Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate.

## Effect of haloperidol, haloperidol+5-$HT_2$ antagonists, olanzapine and Y-931 on the GAD mRNA expression levels.

The effect of haloperidol, olanzapine and Y931 on GAD67 mRNA expression and effect of $5HT_2$ antagonists to modulate haloperidol induced changes in different areas of basal ganglia are shown in table 23 and Fig. 14 and 15. In this experiment, GAD 67 mRNA levels were significantly lowered after treatment with Y931 and olanzapine but not with haloperidol in the globus pallidus as compared to vehicle controls. The expression level of GAD67 mRNA was significantly less after treatment with haloperidol than that with Y931. Interestingly, although olanzapine also decreased GAD 67 mRNA expression in level in this region, the expression level of GAD mRNA after treatment with Y931 was considerably lower than that of olanzapine. The pretreatment with SB242084 (SB2), the selective $5HT_{2C}$ receptor antagonist, but not SB200464 (SB1), reduced GAD 67 mRNA expression level in the lateral globus pallidus compared to controls. Treatment with olanzapine and Y931 also decreased GAD67 mRNA expression level in this region. In the caudate putamen, substantia nigra pars reticulata and in the lateral part of substantia nigra, no changes were observed in any of the treatment groups (Table 23, fig: 14 &15, page: 121-123).

| Region | Veh/Veh | Veh/Hal | | SB1/Hal | | SB2/Hal | | Olanz | | Y931 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ROD | ROD | %Contr | ROD | %Contr | ROD | %Contr | ROD | %Contr | ROD | %Contr |
| LGP | .311±0.02 | .283±0.01 | 90.8±3.4 | .260±0.01 | 84.8±3.5 | .23±0.02 | 74.3±5.8* | .25±0.3 | 80.7±10* | .2±0.02 | 68.0±7.8* # |
| CPu | .391±0.03 | .36±0.02 | 92.07±5.1 | .375±0.02 | 95.9±5.1 | .384±0.02 | 98.34±5.1 | .382±0.2 | 97.7±5.1 | .33±0.02 | 84.4±5.1 |
| SNr | .377±0.01 | .333±0.02 | 88.3±5.86 | .337±0.021 | 89.4±5.7 | .319±0.275 | 84.6±7.3 | .323±0.02 | 85.7±4.6 | .356±0.01 | 94.4±2.8 |
| SNL | .461±0.41 | .48±0.02 | 104.6±4.8 | .469±0.035 | 101.7±7.6 | .463±0.047 | 100.4±10.1 | .498±0.023 | 108.±5.1 | 043±0.04 | 93.1±9.3 |

Table 23. Results of in situ hybridisation showing the effects of haloperidol (1mg/kg) and haloperidol with SB (10 mg/kg) or SB2 (10 mg/kg) on GAD 67 mRNA expression level three hours after antipsychotic drug treatment, in comparison to the effect of Olanzapine (10 mg/kg) or Y931 (10 mg/kg) in the lateral globus pallidus (LGP), caudate putamen (Cpu), substanstia nigra pars reticulata (SNr) and lateral substantia compacta (SNL).

Data shown as mean optical density (ROD) ±SEM and mean percentage of vehicle control (control=100%, n=6-8 per group). Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate. *$p<0.05$ compared to respective vehicle; # = significantly lower than haloperidol $p<0.05$ compared to haloperidol.

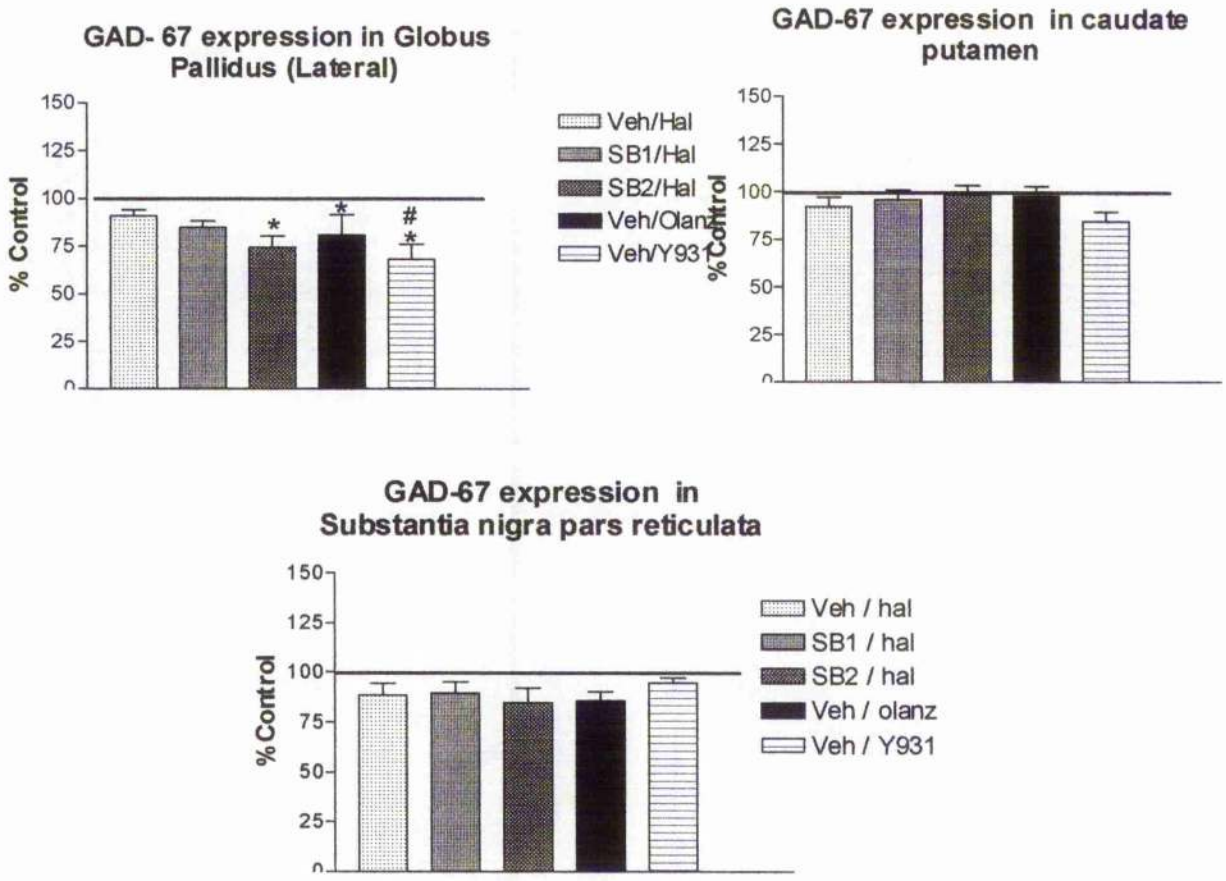


Fig 14. Effect of hal, hal+SB1, hal+SB2, olanz and Y-931 on GAD 67 mRNA expression level in globus pallidus (LGP), caudate putamen (Cpu) and substantia nigra pars reticulata (SNR),three hours after APD treatment. Data shown as mean optical density (ROD) ±SEM and mean percentage of vehicle control (control=100%, n=6-8 per group). Data was analysed by one way ANOVA followed by LSD multiple range tests where appropriate. *$p<0.05$ compared to respective vehicle. # = significantly lower than haloperidol $p<0.05$

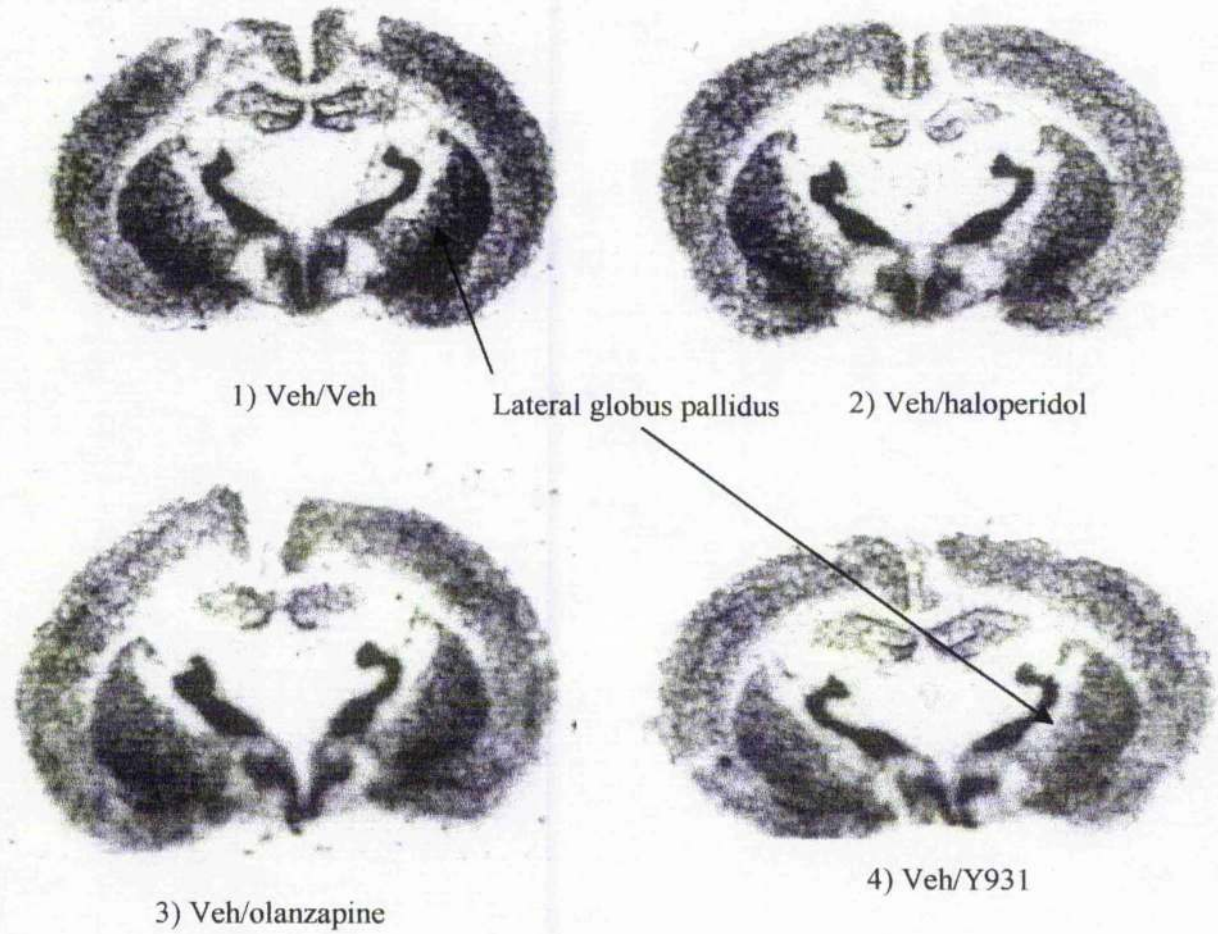


Fig.15. In situ hybridisation autoradiographic localization of GAD 67 mRNA expression in rat brain at the level of globus pallidus (LGP) and caudate putamen (Cpu) (Level-2). Rats were treated with vehicle-1 (0. 5%HPMC), haloperidol –2 (1mg/kg), olanzapine-3 (10 mg/kg) and Y931-4 (10mg/kg).

**Effect of haloperidol, haloperidol+5-$HT_2$ antagonists, olanzapine and Y-931 on the NOS mRNA expression levels.**

The table 24, shows that there was no significant change in the expression level of nitric oxide synthase (NOS) mRNA in the subthalamic nucleus (STN) after three hours treatment with haloperidol, olanzapine or Y-931. Whilst the NOS mRNA expression level appeared lower than the vehicle control after treatment with Y931, this was not significant.

.

| Region | Veh/Veh | Veh/hal | | hal/SB1 | | Hal/SB2 | | Veh/olanz | | Veh/Y931 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ROD | ROD | %Cont | ROD | %Cont | ROD | %Cont | ROD | %Cont | ROD | %Cont |
| STN | .130±030 | .100±.020 | 117±11 | .170±.040 | 132±3.3 | .132±.020 | 101.0±15 | .186±.030 | 140±23 | .116±.020 | 89±12 |

Table 24. Result of in situ hybridisation showing the effects of haloperidol (1mg/kg) and /haloperidol with SB1 (10 mg/kg) or SB2 (10 mg/kg) in comparison to the effect of olanzapine (10 mg/kg) and Y931 (10 mg/kg) on nitric oxide synthase (NOS) mRNA expression level in the subthalamic nucleus (STN), three hours after APD treatment.

Data shown as mean optical density (ROD) ± SEM and mean percentage of vehicle control (control=100%, n=6-8 per group). Data analysed by one way ANOVA followed by LSD multiple range testes where appropriate.

**Effect of haloperidol, haloperidol+5-$HT_2$ antagonists, olanzapine and Y-931 on the grp-75 mRNA expression levels.**

After three hours treatment with haloperidol, olanzapine and Y-931, the expression level of glucose regulating protein (grp-75) mRNA was significantly higher in the subthalamic nucleus (STN) with all three drugs. The expression level of grp 75 mRNA, was largest following treatment with Y931, although it was not significantly greater than any of the other groups. Pretreatment with the serotonin antagonists did not seem to make much change in the pattern of mRNA expression level as compared haloperidol alone.

In substantia nigra pars compacta (SNCD), although there was no significant change in the expression level of grp-67 mRNA, there was a tendency of $5HT_{2C}$ receptor antagonist SB242084 to lower the effect of haloperidol. In this area the expression level of grp75 mRNA was lowest after treatment with Y931 (Table 25, fig 16 &17, page: 127-129).

| Region | Veh/Veh | Veh/hal | | hal/SB1 | | SB2/hal | | olanzapine | | Y931 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ROD | ROD | % Cont | ROD | % Cont | ROD | % Cont | ROD | % Cont | ROD | % Cont |
| STN | .115±.034 | .191±.016 | 166±14.3* | .195±.030 | 169±27.4* | .207±.020 | 180±13.8* | .220±.020 | 188±15.0* | .240±.020 | 207±15* |
| SNCD | .093±.025 | .135±.024 | 146.1±26 | .145±.04 | 156.5±43 | .112±.03 | 120.1±37 | .167±.025 | 179.9±27 | .082±.030 | 88.5±3.1 |

Table 25. Results of in situ hybridisation showing the effects of haloperidol (1mg/kg) and haloperidol with SB1 (10 mg/kg) or SB2 (10 mg/kg) in comparison to the effect of olanzapine (10 mg/kg) and Y931 (10 mg/kg) on grp-75 mRNA expression three hours after APD treatment, in subthalamic nucleus (STN) and substantia nigra pars compacta (SNCD).

Data shown as mean optical density (ROD) ±SEM and mean percentage of vehicle control (control=100%, n=6-8 per group). Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate. *P<0.05 compared to respective vehicle.

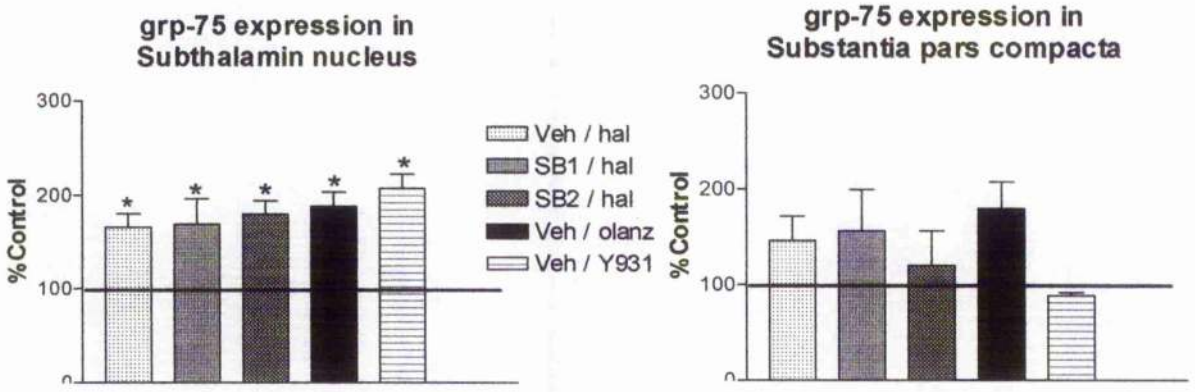


Fig 16 Effect of hal, hal+SB1, hal+SB2, olanz and Y-931 on glucose regulating protein mRNA expression level in subthalamic nucleus (STN) and substantia nigra pars compacta (SNCD), three hours after APD treatment. . Data shown as mean optical density (ROD) ±SEM and mean percentage of vehicle control (control=100%, n= 6 to 8). Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate. *$p<0.05$ compared to respective vehicle.

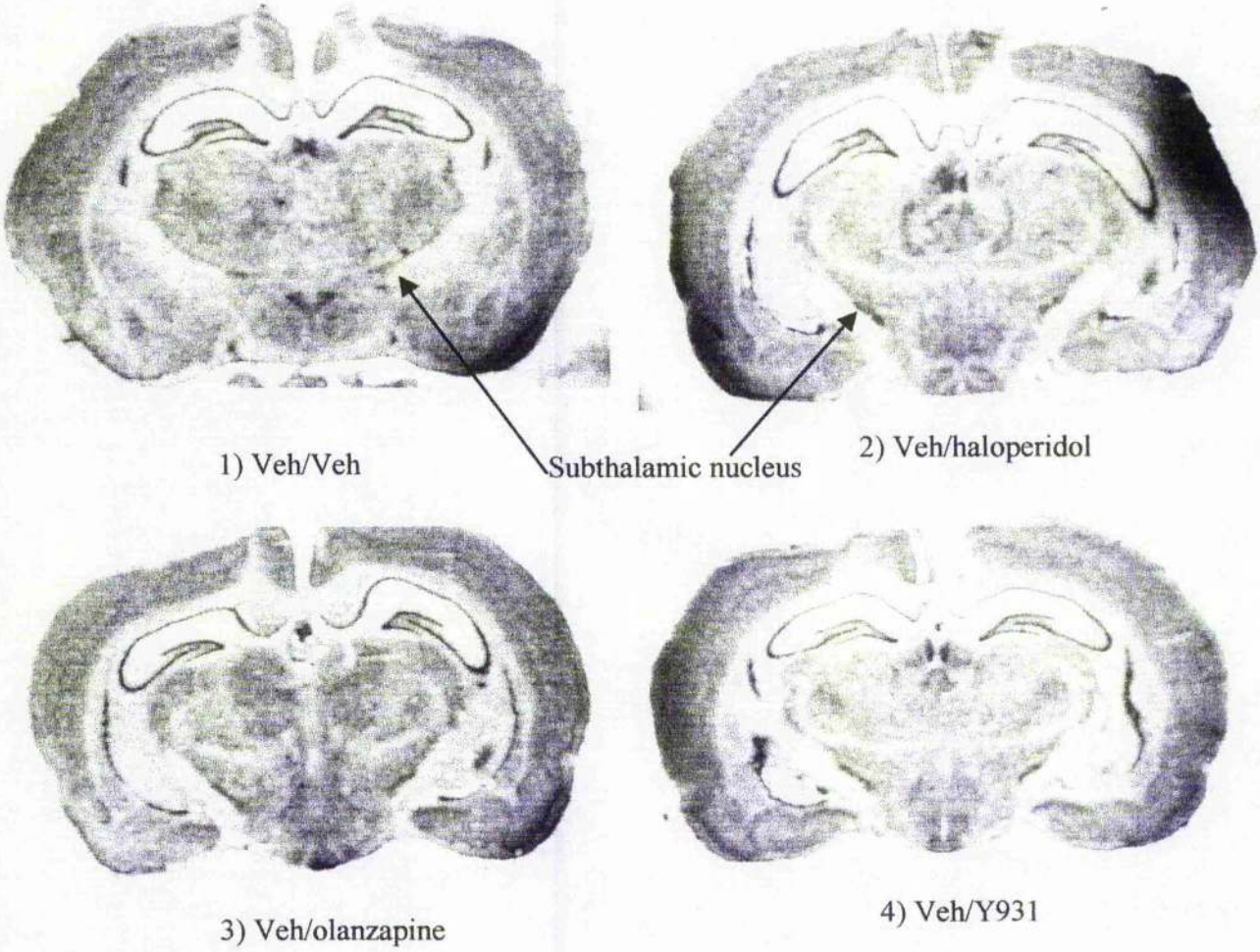


Fig 17. In situ hybridisation autoradiographic localization of grp-75 mRNA expression in rat brain at the level (Level-3) of subthalamic nucleus (STN). Rats were treated with vehicle-1 (0. 5%HPMC), haloperidol -2 (1mg/kg), olanzapine -3 (10 mg/kg) and Y931-4 (10 mg/kg).

## Effect of haloperidol, haloperidol+5-$HT_2$ antagonists, olanzapine and Y-931 on parvalbumin expression levels in different regions of rat brain.

After three hours treatment with haloperidol, olanzapine, and Y931, the expression level of parvalbumin mRNA levels were significantly higher than that of controls in the subthalamic nucleus (STN). The expression level of parvalbumin mRNA was very similar to that of haloperidol after treatment with Y931. Pretreatment with the $5HT_{2B+2C}$ receptor antagonist SB200646 (SB1) significantly attenuated haloperidol induced changes in parvalbumin mRNA expression in this region.

Parvalbumin mRNA expression levels were also higher ($P<0.07$) than that of controls in the lateral globus pallidus after treatment with Y931, but not significant. However none of the other drug-treatments affected parvalbumin expression level in this region.

There were no significant changes in the expression level of parvalbumin mRNA in any other regions examined, such as substantia nigra pars reticulata or caudate putamen. (Table-26 and fig: 18 & 19, page: 131-133)

| Region | Veh/Veh | Veh/Hal | | SB1/Hal | | SB2/Hal | | Olanz | | Y931 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ROD | ROD | % Cont | ROD | % Cont | ROD | % Cont | ROD | % Cont | ROD | % Cont |
| STN | .130±.040 | 0.308±.023 | 236.7±17.6* | 0.224±0.033 | 172±25.6*# | .283±.012 | 217.8 ± 9.3* | .259±.021 | 199.1*±16.4 | .301±0.017 | 232.0±13.3* |
| SNR | .218±.028 | .240±.026 | 110.2 ±11.9 | .22±.022 | 99.4 ± 7.9 | .228±.017 | 104.7 ± 7.9 | .226±.017 | 103.4 ± 7.8 | .265±.028 | 121.6 ±12.7 |
| CPu | .033±.005 | .047±.006 | 142.2 ± 19.9 | .033±.007 | 97.8 ± 20.4 | .030±.006 | 90.9±18.0 | .047±.008 | 140.3 ± 23.2 | .047±.003 | 142.1 ± 9.9 |
| LGP | .092± .110 | .094±.008 | 102.2 ± 9.2 | .086±.012 | 94.2 ±13.6 | .079±.011 | 86.1 ± 12.6 | .107±.011 | 116.6 ± 12.3 | .124±.007 | 135.3 ±7.2 |
| M1L1 | .086±.008 | .111±.010 | 128.6 ± 11.1 | .063±.021 | 73.4 ±24.6 | .081±.018 | 93.4 ± 20.5 | .097±.003 | 112.8 ±3.6 | .085±.009 | 99.0 ±10.0 |
| M1L2 | .131±.013 | .168± .020 | 128.9 ±13.2 | .121±.020 | 92.4 ±19.4 | .164±.018 | 125.1 ± 13.9 | .163±.006 | 124.3 ± 4.6 | .148±.009 | 114.4 = 6.6 |
| M1L3 | .106± .010 | .12±.015 | 113.3 ± 14.5 | .114±.014 | 107.5 ± 13.5 | .131±.016 | 124.2 ± 15.3 | .135±.012 | 127.9 ± 11.3 | .145±.008 | 137.6 ± 7.7 |
| M1L5 | .087±.012 | .115±.014 | 131.9 ± 15.8 | .104±.015 | 119.8 ± 16.7 | .108±.012 | 123.7 ± 13.3 | .120±.008 | 137.8 ± 9.7 | .134± 010 | 154 ±12 |
| M1W | .075±.017 | .106±.015 | 141.6 ± 20.1 | .071±.140 | 94.0 ± 18.1 | .078±.012 | 104.5±15.3 | .094±.007 | 125.4± 9.1 | .10±.009 | 133.0 ± 11.5 |
| M2L1 | .123±.023 | .113±.017 | 91.7± 14.1 | .10±.016 | 81.0 ± 13.3 | .101±.017 | 81.8 ±13.5 | .10±.017 | 81.2 ± 13.9 | .113±.016 | 91.6 ± 12.6 |
| M2L2 | .165±.026 | .147±.016 | 88.6 ± 9.6 | .128±.026 | 77.3 ± 15.6 | .122±.012 | 73.8 ± 7.3 | .171±.015 | 103.1 ± 9.1 | .13 ±.013 | 78.7 ± 7.7 |
| M2L3 | .127±.015 | .108±.013 | 85.3 ± 9.9 | .117±.024 | 92.1 ± 19.0 | .126±.017 | 99.5 ±13.1 | .118± .010 | 92.9 ± 7.9 | .106±.017 | 83.1 ± 13.3 |
| M2L5 | .109±.017 | .10±.012 | 91.3± 11.3 | .97±.018 | 88.6± 16.2 | .104±.013 | 95.4± 11.8 | .099±.008 | 91.1 ±7.5 | .098±.006 | 89.2 ± 5.9 |
| M2W | 0097±.017 | .109±.017 | 112.6 ± 10.1 | .093±.017 | 95.5± 17.1 | .101±.014 | 103.3 ± 14.0 | 0101±.010 | 103.6 ± 10.8 | 0134±.014 | 138 ±14.1 |

Table.26. Results of in situ hybridisation showing the effects of haloperidol (1mg/kg) and haloperidol with SB1 (10 mg/kg) or SB2 (10 mg/kg) to compare the effect of olanzapine (10 mg/kg) and Y931 (10 mg/kg) on parvalbumin mRNA expression three hours after APD, in subthalamic nucleus (STN), substantia nigra reticulata (SNR), striatum ( DLCPu & VMCPu) and cortical areas [Motor cortex- 1 Layer 1, Layer 2, Layer 3, Layer 5 and motor cortex - 1 whole area (M1W); motor cortex- 2 Layer 1, Layer 2, Layer 3, Layer 5 and motor cortex -2 whole area (M2 W) ]. Data shown as mean optical density (ROD) ± SEM and as mean percentage of vehicle control=100%, n=6-8 per group. Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate. *P<0.05 compared to respective vehicle. # = significantly lower than haloperidol.

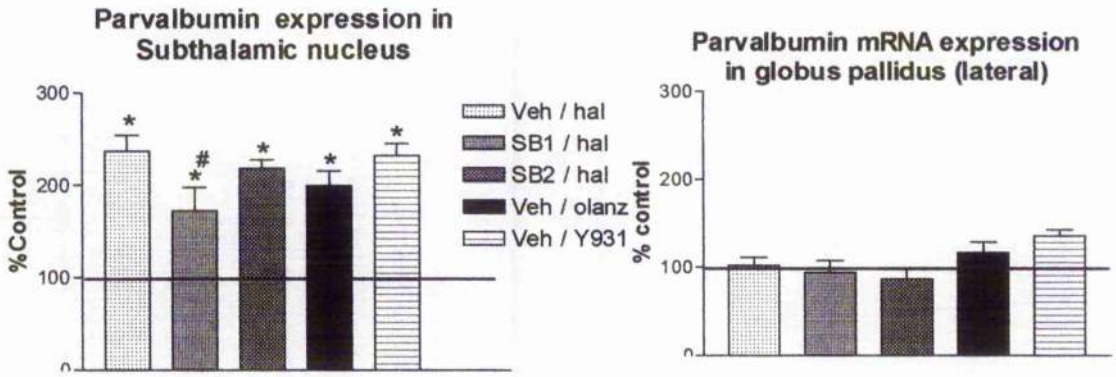


Fig 18. Effect of haloperidol, haloperidol+5HT$_2$ receptor antagonists, olanzapine and Y-931 on of Parvalbumin mRNA expression levels in subthalamic nucleus (STN) and in globus pallidus (LGP),three hours after APD treatment. Data shown as mean optical density (ROD) ±SEM and mean percentage of vehicle control (control=100%, n = 6 to 8 per group). Data analysed by one way ANOVA followed by LSD multiple range tests where appropriate. *$p<0.05$ Compared to respective vehicle; # = significantly lower than haloperidol and Y931.

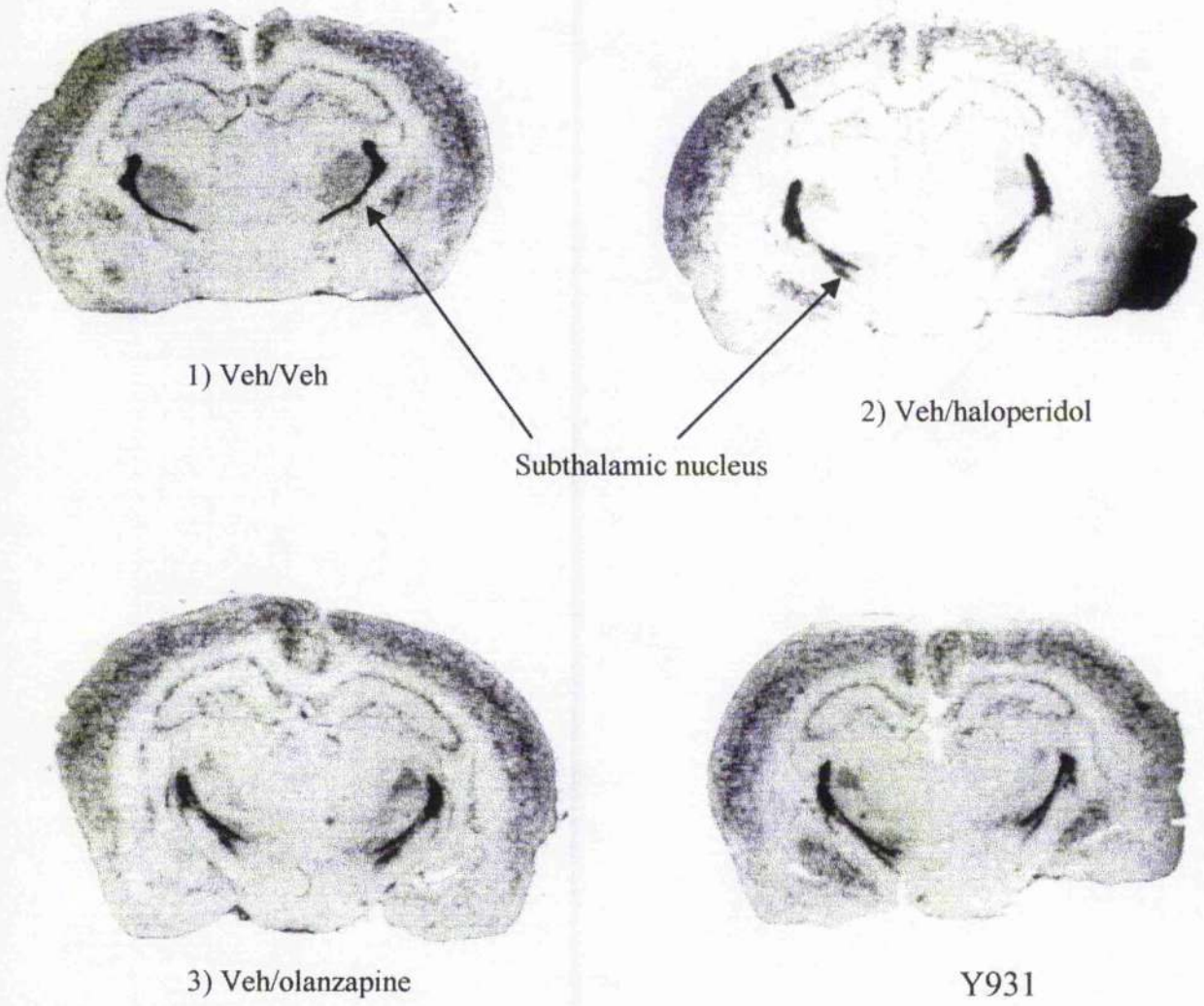


Fig. 19. In situ hybridisation autoradiographic localization of parvalbumin mRNA expression in rat brain at the level of subthalamic nucleus (Level-3). Rats were treated with vehicle 1 (0. 5%HPMC), haloperidol -2 (1mg/kg), olanzapine -3 (10 mg/kg) and Y931-4 (10 mg/kg).

## 4.2.3.Summary of in situ hybridisation experiments, three hours after antipsychotic drug treatment.

There were no drug-induced significant changes in fra-2 mRNA expression in any of the Brain regions examined in this experiment after treatment with haloperidol (1mg/kg), olanzapine (10mg/kg) and Y931 (10 mg/kg). Nevertheless all three drugs showed a tendency to increase fra-2 expression in the dorsolateral caudate putamen and this was highest after treatment with Y931. There was also a tendency for all three drugs to reduce fra-2 mRNA expression in the ventromedial caudate putamen, with olanzapine being most effective in this respect.

The result after situ hybridisation showed that GAD 67 mRNA were significantly lowered after treatment with Y931 and olanzapine in the globus pallidus. After treatment with Y931, the GAD67 mRNA was also significantly lower than that of haloperidol. Haloperidol alone did not change GAD-67 mRNA expression level in the globus pallidus or caudate putamen. However, pretreatment with SB2 ($5HT_{2C}$ antagonist, SB 242084) resulted in a significant decrease of GAD67 mRNA in the lateral globus pallidus in haloperidol treated animals.

The GAD67 mRNA level was not changed in other brain areas examined after treatment with all three drugs.

There was no significant change in the expression level of NOS mRNA level three hours after treatment with haloperidol, olanzapine or Y931. From this result, it could be concluded that nitric oxide synthase (NOS) is not a good marker gene for our purpose.

Three hours after treatment with haloperidol, olanzapine, and Y-931, the expression level of glucose regulating protein (grp-75) was significantly increased in the subthalamic nucleus with all three drugs as compared to controls. Treatment with Y931 produced highest expression of grp mRNA, although this was not significantly greater than with

haloperidol or olanzapine. Pretreatment with the serotonin (2B / 2C) antagonists did not influence the antipsychotic drug-induced changes in the mRNA expression patterns.

After treatment with the three antipsychotic drugs, the expression level of parvalbumin mRNA was significantly higher than that of controls in the subthalamic nucleus (STN). Pretreatment with the 5-$HT_{2B+2C}$ antagonist (SB200646) did lower the effect of haloperidol significantly, resulting in an expression level very similar to that of olanzapine. However, pretreatment with the 5-$HT_{2C}$ selective receptor antagonist SB242084, did not significantly alter the effects of haloperidol.

In the lateral globus pallidus (LGP), the expression level of parvalbumin mRNA did not change with haloperidol or olanzapine treatment in comparison to controls. But after treatment with Y931, it approached significance compared to controls ($P<0.07$) and appeared to reduce compared to haloperidol alone ($p<0.052$).

Although there were no significant changes in the level of parvalbumin expression levels in the motor cortex areas after any of the antipsychotic drug treatments, pretreatment with the $5HT_{2C}$ serotonin receptor antagonist SB200646 appeared to reduce the effect of haloperidol in M1L1 and M1L2 considerably.

The results of grp 75, GAD67 and parvalbumin might indicate that Y931 affects the pallido-subthalamic pathway of the basal ganglia. This effect of Y931 is similar to that of haloperidol indicating that it might have the inclination to cause EPS.

## 4.3.Summary tables of results

### Results after forty-five minutes: IEG mRNA expression in different areas of rat brain (1st experiment).

| Brain area | Zif268 | | c-fos | |
|---|---|---|---|---|
| | hal | Y931 | hal | Y931 |
| Cg1 | ↑* | ↔ | ↑* | ↔ |
| Cg2 | ↑* | ↔ | ↔ | ↔ |
| Motor-cortex 1 Layer-1 | ↔ | ↔ | ↔ | ↔ |
| Motor cortex 1 Layer-2 | ↑* | ↔ | ↔ | ↔ |
| Motor cortex-2 Layer-1 | ↔ | ↔ | ↔ | ↔ |
| Motor Cortex-2 Layer-2 | ↔ | ↔ | ↑* | ↔ |
| Sensory motor cortex-1 Layer-1 | NM | NM | ↓* | ↓* |
| Sensory motor cortex-1 Layer-2 | NM | NM | ↓* | ↓* |
| RSA1 | NM | NM | ↔ | ↔ |
| RSA2 | NM | NM | ↓* | ↓* |
| Ventro-Medial-Caudate-Putamen | ↑* | ↑* | ↑* | ↔ |
| Dorso-Lateral-Caudate-Putamen | ↑* | ↑* | ↑* | ↑* |
| AcbSh | ↔ | ↔ | ↔ | ↔ |
| AcbC | ↑* | ↑* | ↔ | ↔ |

Table 27. Summary of changes in the gene mRNA expression levels produced by the acute treatment with haloperidol (hal) and Y931.

↑* Indicates significant increase in gene expression compared to vehicle control
↓* Indicates significant decrease in gene expression compared to vehicle control
↔ Indicates not significantly different from vehicle control
NM Indicates not measured

**Results after forty-five minutes: IEG m RNA expression (2nd experiment).**

| Brain area | Zif-268 | | | c-fos | | |
|---|---|---|---|---|---|---|
| | hal | olanz | Y931 | hal | olanz | Y931 |
| Cg1 | ↔ | ↔ | ↔ | ↔ | ↔ | ↔ |
| Cg2 | ↔ | ↔ | ↔ | ↔ | ↔ | ↔ |
| Motor-Cortex-1 Layer1 | ↔ | ↔ | ↓* | ↔ | ↔ | ↔ |
| Layer-2 | ↔ | ↔ | ↓* | ↔ | ↔ | ↔ |
| Layer-3 | ↔ | ↓* | ↓* | | ↔ | ↔ |
| Layer-5 | ↔ | ↓* | ↓* | ↔ | ↔ | ↔ |
| Motor cortex-2 Layer-1 | ↔ | ↔ | ↓* | ↔ | ↔ | ↔ |
| Layer-2 | ↔ | ↓* | ↓* | ↔ | ↔ | ↔ |
| Layer-3 | ↔ | ↓* | ↓* | ↔ | ↔ | ↔ |
| Layer-5 | ↔ | ↓* | ↓* | ↔ | ↔ | ↔ |
| Sensory motor cortex-1 Layer-1 | NM | NM | NM | NM | NM | NM |
| Sensory motor cortex-1 Layer-2 | NM | NM | NM | NM | NM | NM |
| RSA1 | NM | NM | NM | NM | NM | NM |
| RSA2 | NM | NM | M | NM | NM | NM |
| Dorso-Medial-Caudate-Putamen | ↔ | ↔ | ↔ | ↑* | ↑* | ↑* |
| Ventro-Lateral-Caudate-Putamen | ↔ | ↔ | ↔ | ↑* | ↔ | ↑* |
| AcbSh | NM | NM | NM | NM | NM | NM |
| AcbC | NM | NM | NM | NM | NM | NM |

Table 28. Summary of changes in the IEG mRNA expression levels produced by the acute treatment with haloperidol (hal), olanzapine (olanz) and Y931 for the second experiment.

↑* Indicates significant increase in gene expression compared to vehicle control

↓* Indicates significant decrease in gene expression compared to vehicle control

↔ Indicates not significantly different from vehicle control

NM Indicates not measured

**Results after three hours APD treatment: late onset gene m RNA expression in deferent areas of basal ganglia.**

| | Fra-2 | | | | | GAD-67 | | | | | Grp-75 | | | | | Parvalbumin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | hal | SB1 +hal | SB2 +hal | olan | Y-931 | hal | SB1 +hal | SB2 +hal | olan | Y-931 | hal | SB1 +hal | SB2 +hal | olan | Y-931 | hal | SB1 +hal | SB2 +hal | olan | Y-931 |
| DLCPu | ↔ | ↔ | ↔ | ↔ | ↔ | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE |
| VMCPu | ↔ | ↔ | ↔ | ↔ | ↔ | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE |
| Cpu | NE | NE | NE | NE | NE | ↔ | ↔ | ↔ | ↔ | ↔ | NE | NE | NE | NE | NE | ↔ | ↔ | ↔ | ↔ | ↔ |
| LGP | NE | NE | NE | NE | NE | ↔ | ↔ | ↓* | ↓* | ↓* | NE | NE | NE | NE | NE | ↔ | ↔ | ↔ | ↔ | (↑) |
| STN | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | ↑* | ↑* | ↑* | ↑* | ↑* | ↑* | ↑* | ↑* | ↑* | ↑* |
| SNCD | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE | ↔ | ↔ | ↔ | ↔ | ↔ | NE | NE | NE | NE | NE |
| SNR | NE | NE | NE | NE | NE | ↔ | ↔ | ↔ | ↔ | ↔ | NE | NE | NE | NE | NE | ↔ | ↔ | ↔ | ↔ | ↔ |

Table 29. Summary of gene mRNA expression levels produced after three hours treatment of haloperidol (hal), olanzapine (olan) and Y931with or without pretreatment with $5HT_2$ antagonists SB1 or SB2.

↑* Indicates significant increase in gene expression compared to vehicle control
↓* Indicates significant decrease in gene expression compared to vehicle control
(↑) Indicates approached significance compared to vehicle control and haloperidol
↔ Indicates not significantly different from controls
NE Indicates not expressed

# Chapter 5: Discussion

## 5.1.General Discussion

Each antipsychotic drug has its own specific pharmacological profile ultimately determining its effect on different targets in the brain.

Several new antipsychotic drugs have full antipsychotic action and low motor side effect. Evidence suggests that the therapeutic action of most atypical antipsychotic drugs cannot be attributed to the action of any single receptor system. It is more likely to be due to the combined activity at some or many neurotransmitter receptors (Bymaster et al. 1997) that are involved in the action of the specific antipsychotic drug. Previous work showed that this multi-affinity profile is also the characteristic of Y931 (Morimoto et al. 2002).

The receptor binding profile of Y931 is similar to that of olanzapine, but with a higher affinity to the dopamine $D_2$ receptor. Y931 also has high affinity for several other receptors like olanzapine and clozapine, e.g., $5HT_2$, $5HT_7$, α-adrenoreceptors, muscarinic receptors M1, M2, M3, M4, M5 and histamine H1 receptors. It is believed that EPS is associated with potent $D_2$ antagonist activity of antipsychotic drugs. This receptor site is also the potential site of antipsychotic action of all APDs.

The results from the present IEG studies showed that, in our first study, Y931 produced relatively less induction of zif 268 in the motor cortex than haloperidol. However, in our second study, we tried to use more intricate method of image analysis to monitor the signals in the individual layer of cortex, which failed to confirm this finding. Haloperidol and Y931 both induced zif268 mRNA expression in the accumbens core. After treatment with haloperidol or Y931, the zif-268 and c-fos IEG expression levels were very similar in dorso-lateral and ventro-lateral caudate putamen. Both c-fos and zif-268 mRNA expression levels were significantly enhanced after treatment with haloperidol and Y931. But after treatment with Y931, the expression level of c-fos mRNA was far greater in the dorsolateral caudate putamen, than after treatment with haloperidol. This may reflect Y931's higher affinity for $D_2$ dopamine receptor. But in the ventro-lateral caudate-putamen, the c-fos expression level was increased by haloperidol but not by Y931.

Thus, Y931 induces a similar pattern of change in IEG expression in the striatum to haloperidol. This is consistent with evidence that $D_2$ receptor antagonism is responsible for increases in the striatal IEG expression.

It would therefore be expected that Y931 would cause EPS. Thus our results of IEG expression suggest that the activity of Y931 could not suppress EPS through it's mechanisms inside the striatum.

However, it had been suggested that Y931 produces no or very little EPS (Morimoto et al., 2002).

In our study, pretreatment with the $5HT_2$ antagonists (SB1 and SB2) employed in this study did not affected haloperidol's ability to induce striatal IEG expression. This would imply, any ability of these serotonergic drugs to modify EPS could be somewhere downstream of the striatum.

Part of the reason for this effect could be Y931's very high affinity towards $5HT_{2C}$ receptors. It is believed that $5HT_{2C}$ receptor antagonists may inhibit centrally mediated hypolocomotor response (Kennett 1993; Di Matteo et al., 1999). It is also believed that $5HT_{2C}$ receptor antagonists may attenuate haloperidol-induced catalepsy (Kennett et al, 1997; Meltzer et al., 1996; O'Neill et al., 1999; Reavill et al., 1999; Poyurovsky and Weizman, 1997). The serotoninergic system has been implicated in the regulation of immediate early genes in the striatum. Activation of serotonin $5HT_{2C}$ receptors has been demonstrated to cause a localised expression of immediate early gene c-fos and this induction could be selectively blocked with a $5HT_{2C}$ antagonist (Leslie et al., 1993).

Neither of the $5HT_{2C}$ antagonists in our experiments, showed significant ability to reduce the haloperidol induced striatal IEG expression, hence any ability of these drugs to modify EPS must be downstream of the striatum.

According to the double blind PET study by Kapur and colleagues (2000a, 2001), it was found that the clinical response of antipsychotic drugs (APD) was manifested at 65-70% $D_2$ receptor occupancy and patients with a $D_2$ receptor occupancy >78% with antipsychotic treatment, showed signs of EPS. For example, risperidone becomes effective at a dose of 2mg/day, which is associated with 65% $D_2$ receptor occupancy with very little EPS, while risperidone 6 mg/day leads to a $D_2$ receptor occupancy of >80% and is associated with EPS. With olanzapine, similar efficacy appears to begin at 10 mg / day, a dose that leads to 70% $D_2$ receptor occupancy; while 30-40 mg / day of olanzapine (effective medical dose) results in $D_2$ receptor occupancy >80% and EPS. The threshold of $D_2$ receptor occupancy lies in the range of 65%-70% (Kapur et al. 1999; Kapur et. al. 2000a, 2000b; Farde.et.al.1992). Quetiapine, on the other hand, produced modestly high (58-64%) $D_2$ receptor occupancy, and only transiently, after 2-3 hours of a single dose, and never exceeded 80% and never produces EPS (Kapur et al. 2000b). Thus, the discrete difference between physiologically active dose and therapeutic dose makes a great difference in the expression of EPS, as aiming for the maximum tolerated levels of antipsychotic drugs is the aim of clinical practice. Whilst it appears that $D_2$ receptor occupancy is a factor in the development of EPS, other receptors may also be important.

The high affinity for $5HT_2$ receptors has been receiving much focus in recent years in relation to atypical antipsychotic drugs. It had been suggested that the balanced antagonism between $5HT_2$ receptors and $D_2$ receptors might contribute to a lower EPS incidence (Meltzer et al., 1989,1991; Barnes and Sharp 1999; Bymaster et al., 1996; Kapur et al., 1999; Pilowsky et al 1992; Stockmeier et al 1993). 5-HT neurons innervate both the dopamine cell bodies in the substantia nigra pars compacta and the ventral tegmental area, and their projection terminal fields in the striatum, nucleus accumbens and frontal cortex. Therefore, 5-HT mechanisms are well placed to modulate dopaminergic processes (Wolf et al., 1997). Y931 has high affinity for $5HT_{2C}$ receptors. It has been suggested that $5HT_{2C}$ receptor antagonists attenuate haloperidol induced catalepsy (Reavill et al., 1999; Meltzer et al., 1996; Wood et al., 2001). But this ability of $5HT_{2C}$ receptor antagonist was not evident in our catalepsy experiments. Pretreatment with the two most robust receptor antagonists (SB200646, a mixed $5\text{-}HT_{2C+2B}$ receptor

antagonist and SB242084, a selective 5-$HT_{2C}$ receptor antagonist) did not reverse the haloperidol induced catalepsy significantly although there appeared to be a modest effect of SB 200646. A similar result was found by other researchers with other potent 5-$HT_{2C}$ antagonists (Bonhaus et al., 1997; Di Matteo et al., 1999; Kennett et al., 1994). Although another group (Kalkman et al., 1998) reported that the $5HT_{2B/2C}$ receptor antagonist SB200646 could not attenuate catalepsy induced by loxapine, a $D_2$ receptor antagonist. And again, another group did find that a $5HT_{2B/2C}$ antagonist SB200646 could attenuate haloperidol-induced catalepsy significantly, while the $5HT_{2B/2C}$ antagonist SB215505 did not do so (Reavill et al., 1999). Although the relative $D_2$ : $5HT_2$ receptor binding affinities, as well as behavioral effects, have been implicated as important to the atypical mechanism of action, the actual degree of receptor occupancy of multiple neurotransmitters achieved during antipsychotic drug treatment may instead be crucial to determining the functional profile of an antipsychotic drug. Also, doses of haloperidol producing 25% striatal and limbic $D_2$ receptor occupancy will not produce significant serotonergic, adrenergic, histaminergic or muscarinic receptor occupancy (Kinon and Lieberman, 1996).

In the behavioural study, our initial result showed that with 1mg/kg of haloperidol treatment, at 60 mins post APD, there was no emergence of catalepsy, but these animals were cataleptic at a later time. However, the fact that not all animals treated with haloperidol developed catalepsy led us to further investigate the effects of SB 200646 against a dose of haloperidol that produced reliable catalepsy. In our second experiment for haloperidol induced catalepsy, 2mg/kg haloperidol effectively produced catalepsy in all animals in the group at 90 mins onwards. This confirmed the fact acknowledged by other researchers (Kapur et al., 2000a, 2000b) that catalepsy (a marker for EPS) induced by haloperidol and other typical high potential antipsychotic drugs, is probably dose dependent.

The results of our first behavioural experiments were different after treatment with olanzapine and Y931 from what was expected. After 60 mins of APD treatment little catalepsy was induced by olanzapine and Y931 at this time point, although the Y931 treated rats appeared somewhat sedated. This may reflect olanzapine's very low affinity

for α-2 receptors and H1 receptors as compared with Y931 and also reflect its less sedative actions. After 120 mins the behaviour of animals treated with Y931 changed. The animals behaved differently with different tests at this time point. In the block test, more of the Y931 treated animals appeared cataleptic (sedated), although in the grid test this was not apparent. One possible explanation of these differences is that the animals were experiencing sedation rather than catalepsy and that this was more apparent in one test than the other. Sedated animals would be drowsy and may show a greater propensity to stay on the block. The high affinity of Y931 for H1 receptors would be consistent with a sedative effect.

In our second set of behavioural experiments using the bar test for assessment of catalepsy treatment with Y931, the animals appeared sedated (cataleptic?) even after 180 mins. And 90 mins onwards, after treatment with olanzapine 50% of the animals also appeared cataleptic (sedated?).

Thus, the ability of these tests to discriminate between cataleptic and sedative effects was not totally clear. In future experiments it might be possible to determine if the catalepsy measures were being confounded by sedation by assessing the animal's response to a sensory stimuli. Thus if an animal was experiencing sedation it would not be expected to show a startle response to a loud noise, whereas a cataleptic animal would be expected to respond to that.

In summary, the results of the catalepsy tests concluded that the addition of a $5HT_{2C/2B}$ receptor antagonist may show a weak ability to attenuate haloperidol induced catalepsy. The selective $5HT_{2C}$ antagonist (SB2, SB242084) on the other hand, was ineffective. Previous studies regarding the effects of mixed $5HT_{2A/2C}$ (SB 200646A) and $5HT_{2C/2B}$ (SB200646) receptor antagonists on dopamine $D_2$ receptor-mediated catalepsy are also inconsistent. SB206553 (a $5HT_{2C/2B}$ receptor antagonist with higher affinity for $5HT_{2C}$receptors than SB200646) also showed variable response. The results varied between attenuation (Reavill et al., 1999), inhibition, (Kennett et al., 1994; 1996; Lucas et al., 1997), no effect (Wadenberg, 1992) or potentiation (Elliott et al., 1990). Whilst

inconclusive, because of the possible confounding effects of sedation of Y931, it is possible that the higher receptor affinity of Y931 for $5HT_{2C/2B}$ receptors may contribute to its lack of catalepsy.

It had been suggested that $D_1$ receptor stimulation is necessary for the expression of postsynaptic $D_2$ receptor mediated functional responses and normal motor behaviour (Gershanik et al., 1983). But this relationship is not mutual since $D_2$ receptor activation is not necessary for $D_1$mediated motor responses (White et al., 1988). It had also been suggested that $D_2$- antagonist-induced catalepsy is mainly mediated via activation of strio-pallidal GABA pathways whereas $D_1$ receptor antagonist induced catalepsy is mainly mediated via increased activity in the nigrothalamic GABA pathways (Ögren S O and Fuxe K, 1988). Therefore alterations of $D_1$ receptor activity may play important roles in the pathophysiology of disorders of DA neurotransmission such as Parkinson's disease and drug-induced Parkinsonism in schizophrenia. Thus the fact that the different doses of haloperidol did not correlate with the degree of IEG expression in the striatum and production of catalepsy, may have some relation to this $D_1$/ $D_2$ interaction and this relationship needs further investigation. Although there was a reduction in the GAD67 expression level after treatment with Y931 and also after pretreatment of haloperidol with SB2 (SB242084), SB242084 did not reduce haloperidol-induced catalepsy. Like clozapine Y931 is also a mixed $D_1$/ $D_2$ antagonist. But, while clozapine is a weak $D_1$/ $D_2$ antagonist causing partial blockade of both receptor types, Y931 is a much stronger $D_1$/ $D_2$ antagonist similar to that of haloperidol. Thus we could not conclude that reduction of pallidal GAD67 expression with Y931 would play a role in attenuating EPS.

Studies related to Parkinson's disease suggests that the neurochemical phenotype of the parkinsonian basal ganglia includes an increase in the neuropeptide enkephalin mRNA expression in the striatum and a decrease in the striatal substance P mRNA expression with increased cytochrome oxidase 1 (CO 1) mRNA in the STN. This suggests an increased STN neural activity. There is also increased glutamate decarboxylase-67 (GAD 67) mRNA expression in the globus pallidus (Salin et al., 1997). Not very much is known about any

other neuropeptide in relation to basal ganglia activity after antipsychotic drug treatment and occurrence of EPS.

In our experiment for marker genes, neither haloperidol nor Y931 altered the expression level of GAD 67, grp-75 or NOS mRNA in the discrete regions of basal ganglia, e.g., subthalamic nucleus and / or substantia nigra, after 45 mins of antipsychotic drug treatment. Despite the change in c-fos and zif 268 mRNA at this time point, there was no haloperidol-induced catalepsy at this time point either. Hence these results are consistent with the idea that these genes are affected more slowly than IEGs and these genes were not good markers of regional activity at the time point studied here.

The effect of haloperidol, olanzapine and Y931 on the marker genes were examined again, three hours after the drug treatment, together with pretreatment with the serotonin $5HT_{2C}$ antagonists 15 mins before haloperidol treatment. The results showed specific alterations of mRNA expression levels with some of the marker genes.

GAD67 mRNA was expressed, at significantly lower levels than the controls, in the lateral globus pallidus (LGP) after treatment with olanzapine and Y931, but not with haloperidol. These results are very similar to that of $D_2$ receptor deficient mice, observed by Murer et al. (2000) and Sakai et al., (2001). In haloperidol treated animals, the level of GAD67 mRNA expression (three hours after treatment) was significantly lowered in the animal group that was pretreated with serotonin antagonist $5HT_{2C}$ receptor (SB 2420084), bringing it to the same level as that with Y931 and olanzapine. This could be the evidence that reduced pallidal GAD67 expression acts to attenuate EPS initiated by $D_2$ dopamine receptor antagonism in the striatum. The decreased pallidal neuronal activity, which presumably reflects decreased GAD67 expression, may result in abnormal increased afferent signal from STN to SNR.

From the study of basal ganglia models and the study of Parkinson's disease many authors deduced that the reduced pallidal GAD67 expression acts to attenuate the tendency towards EPS initiated by $D_2$ dopamine receptor antagonism in the striatum as

decreased GABAergic activity in the pallido-subthalamic pathway is the major factor associated with EPS (Delfs et al., 1995; Vila et al., 1997; Schoeder and Schneider, 2001). In our study, GAD67 mRNA expression was not different from control with haloperidol alone either 45minutes or three hours after treatment. Pretreatment with the 5-$HT_{2C}$ antagonist SB242084 (SB-2) significantly lowered the haloperidol induced GAD67 mRNA expression level. This result is very similar to GAD67 mRNA expression level after treatment Y931 and olanzapine. The GAD67 mRNA expression after treatment with Y931 (three hours after treatment) was also significantly lower than that of haloperidol alone. This may suggest that the change by Y931 could be due to its high affinity for $5HT_{2C}$ receptor. Thus, this reduced pallidal GAD67 expression could be the mechanism that may make Y931 less liable towards EPS initiated by $D_2$ dopamine receptor antagonism in the striatum. And yet there was no clear relationship between the drugs or drug combinations used in this study to reduce catalepsy. Further study is needed to clear this anomaly.

Nitric acid synthase (NOS) mRNA expression did not change with our drug treatment even three hours after drug treatment in any areas of rat brain. But grp75 mRNA expression was increased in subthalamic nucleus by haloperidol alone and also, in combination with either of the serotonin antagonists, and also with Y931 and olanzapine. Dopamine or dopamine agonists increase glucose utilisation and discharge frequency of STN neurons. Our findings are in line with the findings that in drug induced parkinsonism or EPS, STN becomes hyperactive as a result of disinhibition via GPe in the indirect pathway (Henderson et al., 1999; Vila et al., 1997, 2000; Robledo and Féger, 1990). But there was no correlation between grp75 expression in STN and the degree of catalepsy detected in the animals. Other investigators also reported decreased, increased or even mixed effects of dopamine on the frequency of discharge of neurons of the STN (Campbell et al., 1985). Such different effects could also be of a different action of dopamine on $D_1$ and $D_2$ receptor subtypes- which are known to be in the Striatum, SNr (Dawson et al., 1987) and STN (Brown et al., 1979; Marters et al., 1985). Y931 has high affinity for the $D_1$ receptors. As previously mentioned, it had been suggested that $D_2$-receptor antagonist induced catalepsy is mediated via increased activity of striato-pallidal

GABA pathways whereas D1-receptor antagonist induced catalepsy is mainly mediated via increased activity in the nigrothalamic inhibitory (GABA) pathways. Thus different neuronal networks containing different receptor populations and transmitters, may be involved in the catalepsy caused by $D_2$ and $D_1$ receptor antagonists (Ögren S O and Fuxe K, 1988; Morelli and Chiara, 1985). In our study, whilst all three antipsychotic drugs induced increased grp75 mRNA expression in the STN, only haloperidol produced clear catalepsy in all animals in this treatment group, which may reflect this aspect of striatum, SNR and STN nucleus.

GABAergic neurons can be defined by the presence of one of three essentially non-overlapping calcium binding proteins (CBPs): parvalbumin, calretinin and calbindin (Reynolds et. al., 2001; Marshall et al. 1998). Reports from post mortem tissue analysis of schizophrenic patients revealed that parvalbumin (PV), but not calretinin containing cells were significantly diminished in schizophrenia (Nitsch et al., 1989). Thus parvalbumin appears to be a very important marker for GABAergic neuronal subtypes.

Our experiment showed that the expression level of parvalbumin mRNA increases significantly relative to the control, after treatment with all three drugs: haloperidol, olanzapine and Y931 in the subthalamic nucleus (STN). Pretreatment with the selective $5HT_{2C}$ receptor antagonist SB 242084 with haloperidol lowered the effect of haloperidol slightly. It is interesting to note that the result after pretreatment with SB200646 ($5HT_{2C+2B}$) did lower the effect of haloperidol considerably, which was more like that of olanzapine, where as the result of pretreatment with SB242084 was more like that of Y931.

Once again, as with grp75 mRNA expression, there did not appear to be a correlation between the extent of drug-induced parvalbumin expression and the degree of catalepsy detected.

There was no change in the mRNA expression level of the nitric oxide synthase (NOS) in the cerebral cortex, STN, striatum or nucleus accumbens with any of the drugs at any time points (45 mins or 3 hours after acute treatment). Again, this contrasts with the

reports that NOS mRNA expression is a sensitive indicator of the activity of STN (Nisbet et al., 1994; Bel et al., 2000; Shinde et al., 2000).

The mRNA encoding $5HT_{2C}$ receptors is highly expressed in the subthalamic nucleus (Eberle-Wang et. al., 1997). The subthalamic nucleus receives a cortical innervation in the rat. This direct cortico-subthalamic projection is excitatory and glutamatergic. There is also dopaminergic innervation of the frontal cortex of rat brain. The neurons in the frontal cortex express $D_1$ and $D_2$ receptors. Since the activation of these dopaminergic receptors decreases the glutamatergic transmission, it could be hypothesised that the $D_2$ antagonism in these area leads to increased activity of the excitatory cortical neurons projecting to the subthalamic nucleus (Hassani et al., 1996).

The STN projects to, and influences the activity of major output nuclei of basal ganglia: globus pallidus (GP) and substantia nigra reticulata (SNr). The main neurotransmitter used by the STN is glutamate. As described above, it is rich in PV positive neurons, which is usually a GABA marker. The PV positive sectors of STN appear related to the GP and cerebral cortex. Subthalamic lesions alleviate parkinsonian symptoms in animals showing the importance of the STN in the control of motor behaviour (Aziz et. al., 1991). The presence of both mRNA and binding sites for $5HT_{2C}$ receptors in the STN suggests that these receptors are expressed by intrinsic subthalamic neurons. The major target areas of these neurons are the pallidum and SNR, the main output structures of the basal ganglia.

It had been proposed that in the STN, $5HT_2$ receptor stimulation is associated with an increased firing rate of the STN neurons. Hence $5HT_{2C}$ receptor antagonism may act to reduce STN firing that has been elevated by striatal $D_2$ receptor antagonism. From our results it is evident that the drug SB200646 (a selective $5HT_{2C/2B}$ antagonist) and the drug SB242084 (a selective $5HT_{2C}$ antagonist) were not as effective as expected in restoring altered gene expression following haloperidol treatment. SB 200646 is a selective $5\text{-}HT_{2C/2B}$ receptor antagonist with pki value of 6.9 for serotonin-$_{2C}$ receptors (Forbes et al., 1995) and with pki value of 7.5 for serotonin $2_B$ receptors. SB200646 has

50-fold selectivity for 5-$HT_{2C}$ and 5-$HT_{2B}$ over 5-$HT_{2A}$ receptors (Kennett et al., 1994). Where as, SB 242084 is a selective 5-$HT_{2C}$ receptor antagonist with pki value 9.0 for serotonin $_{2C}$ receptors. It has 100 and 158-fold selectivity over the 5-$HT_{2B}$ and 5-$HT_{2A}$ receptor subtypes respectively. Thus SB242084 can be used to evaluate the effect of selective blockade of 5-$HT_{2C}$ receptors. (Di Matteo et al., 1999). The results indicate that $5HT_{2C+2B}$ antagonist had more influence on the effect of $D_2$ antagonism on some of the regional marker genes of the basal ganglia.

The antagonism of $D_2$ dopamine receptors, or dopaminergic depletion in the striatum, is predicted to cause increased activity of the GABA-ergic striatal neurons projecting to the globus pallidus. This should cause increased GABA release. But if the GABA-ergic pallidosubthalamic neurons were then inhibited, there would then be an increase in the subthalamic neurons discharge rate. However, a subthalamo-pallidal projection influences the pallidal neurons, which express GAD67 mRNA. Thus the activity of the pallidal neurons may also be regulated by the subthalamic nucleus (Hassani et. al., 1996). So, the activity of the pallidal neurons may also be regulated by the subthalamic nucleus, which is assigned as the area of convergence of inhibitory and excitatory inputs on the pallidum (Hassani, 1996; Parent, 1995), not just a relay station for the indirect pathway. Thus it could be that alteration of both serotonergic and dopaminergic neurotransmission may be needed to reduce the motor symptoms in the EPS. Some researchers (Eberle-Wang et al., 1996) showed that there could be dopaminergic / serotonergic interactions in the STN. Thus EPS produced by some antipsychotic drugs could have resulted from direct or indirect alterations in the activity of subthalamic neurons respectively. It is believed that the hyperactivity of these glutamatergic STN projection neurons to the globus pallidus (GPi) may be a cause of hypoactive movement disorder and extrapyramidal side effects of the neuroleptic drugs. Thus the results for grp-75 and parvalbumin expression levels in the STN suggest that, our hypothesis that Y931 may suppress EPS by its ability to influence indirect pathway via its higher affinity for $5HT2_C$ receptor at a site out side striatum, is unlikely to be correct.

In addition to the GABAergic inhibitory projections from the striatum, GPe receives a dense glutamatergic excitatory input from the STN. This double innervation suggests that the activity of GPe neurons results in the counter balancing of the activity of these two convergent pathways: the inhibitory pathway arising from the striatum and the excitatory input from the STN, producing normal or increased activity of GPe neurons (Levy et al., 1997). The STN may not be the only excitatory input responsible for the activation of GPe neurons. Other excitatory inputs could maintain or increase the activity of the GPe in the normal state or Parkinsonian state, e.g., the parafascicular nucleus of the thalamus (Pf nucleus), which sends projection to the GPe. The STN also receives projections from glutamate-excitatory afferent projections from the cerebral cortex, the glutamate-excitatory projections from the parafascicular nucleus of the thalamus (Pf nucleus) (Parent et al., 1995; Mouroux et al., 1993). The cerebral cortex and Pf nucleus are glutamatergic and can cause hyperactivity seen in the STN. However, the precise influence, or any crucial role of each of these afferent systems to reduce the drug induced Parkinsonism after treatment with Y931, need to be determined by further study.

## 5.2.Conclusion

Thus from the above discussion, the following conclusions can be made:

According to our first hypothesis, Y931 acts within the striatum to suppress the activation of indirect pathway, despite blocking striatal $D_2$ dopamine receptors. But, haloperidol, olanzapine and Y931 all tend to increase striato-pallidal activity, as assessed from the IEG expression in the striatum. Furthermore, they all also tend to increase STN activity, as assessed by grp75 and parvalbumin mRNA expression level although these changes in gene expression did not correlate with drug induced catalepsy. Thus the reduced EPS liability of Y931 is unlikely to be due to actions in the striatum.

In our second hypothesis, we suggested that Y931 acts outside the striatum to suppress the activation of the indirect pathway and it could be via its relatively high affinity for the $5HT_{2C}$ or muscarinic M3 receptors. Our results do not provide clear support for the hypothesis that either $5HT_{2B}$ and / or $5HT_{2C}$ receptor antagonism suppresses the appearance of EPS. Our results also are not clear about the activity of $5HT_{2C}$ receptors in controlling antipsychotic drug induced catalepsy. We did not manage to explore the possibility of M3 receptor as an appropriate compound was not available at the time. Thus the reduced EPS liability of Y931, if any, may lie with some other feature of the compound.

Our third hypothesis speculates that Y931 may facilitate and elevate the activity of the direct pathway thus balancing the indirect pathway and control EPS. But our results do not show any direct evidence to this effect.

Y931 increase striato-pallidal activity. It very strongly increases the activity of subthalamic nucleus, thus increasing activity of the indirect pathway from the striatum to the substantia nigra.

Thus the action of Y931 to suppress EPS due to $D_2$ receptor antagonism in the striatum is likely to be down stream of the substantia nigra. The anatomical and pharmacological locus of Y931 in suppressing EPS, if any, remains to be identified.

How atypical antipsychotics achieve their advantages over conventional antipsychotic agents remains uncertain. Each of these agents has a somewhat distinct profile in terms of specific serotonin and dopamine sub-receptor affinity, and affinity for other neurotransmitters receptors. There is no consensus as to what combination or ratio of receptor activities is essential for the clinical advantage shown. The explanation for the reported low profile of Y931 in experimental models therefore remains to be determined.

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# Appendix:

## Statistics where ANOVA has been significant.

Table no. 8: zif 268

| Region | Degree of reference (df) | F | P |
|---|---|---|---|
| M1L2 | 2,21 | 3.924 | 0.036 |
| M2L1 | 2,21 | 4.386 | 0.026 |
| Cg1 | 2,21 | 6.242 | 0.007 |
| Cg2 | 2,21 | 4.569 | 0.023 |
| DLCPu | 2,21 | 19.648 | 0.000 |
| VMCPu | 2,21 | 17.737 | 0.000 |
| AcbC | 2,21 | 8.370 | 0.002 |

Table no. 9: c-fos

| Region | Degree of reference (df) | F | P |
|---|---|---|---|
| Cg1 | 2,19 | 4.300 | 0.29 |
| M2L2 | 2,19 | 3.950 | 0.037 |
| VMCPu | 2.19 | 3.826 | 0.040 |
| DLCPu | 2,19 | 13.876 | 0.000 |
| RSAL-2 | 2,20 | 4.893 | 0.019 |
| S1L1 | 2.19 | 5.368 | 0.014 |
| S1L2 | 2,17 | 10.677 | 0.001 |

Table no. 10: zif 268

| Region | Degree of reference (df) | F | P |
|---|---|---|---|
| M1L1 | 3,20 | 5.990 | 0.004 |
| M1L2 | 3,20 | 5.608 | 0.006 |
| M1L3 | 3,20 | 5.598 | 0.006 |
| M2L1 | 3,20 | 5.058 | 0.009 |
| M2L2 | 3,20 | 9.254 | 0.000 |
| M2L3 | 3,20 | 4.564 | 0.014 |

Table no. 11: zif 268

| Region | Degree of reference (df) | F | P |
|---|---|---|---|
| M1L1 | 3,17 | 3.687 | 0.33 |
| M1L2 | 3,17 | 4.157 | 0.22 |
| M1L3 | 3,17 | 4.342 | 0.019 |
| M1L5 | 3,17 | 4.822 | 0.013 |
| M2L1 | 3,17 | 3.774 | 0.030 |
| M2L3 | 3,17 | 4.822 | 0.013 |
| M2L5 | 3,17 | 3.421 | 0.041 |
| M1W | 3,17 | 5.110 | 0.011 |
| M2W | 3,17 | 3.121 | 0.053 |

Table no. 12: c-fos

| Region | Degree of reference (df) | F | P |
|---|---|---|---|
| DLCPu | 3,14 | 7.574 | 0.003 |
| VMCPu | 3,14 | 4.575 | 0.020 |

Table no. 23: GAD

| Region | Degree of reference (df) | F | P |
|---|---|---|---|
| LGP | 5,34 | 3.203 | 0.018 |

Table no. 25: grp

| Region | Degree of reference (df) | F | P |
|---|---|---|---|
| STh | 5,33 | 3.191 | 0.019 |

Table no. 26: Parvalbumin

| Region | Degree of reference (df) | F | P |
|---|---|---|---|
| STh | 5,30 | 6.725 | 0.000 |

# References.


Aizman O, Brismar H, Uhlen P, Zettergren E, Levey AI, Fossberg H, Greengard P and Aperia A (2000). Anatomical and physiological evidence for D1 and D2 dopamine receptor co-localization in neostriatal neurons. Nature Neurosci 3, 226-230

Albin R L, Aldridge W, Young A B, and Gilman S (1989) Feline subthalamic nucleus contains glutamate-like but not GABA-like or glycine-like immunoreactivity. Brain Res. 491, 185-188

Albin R L, Young A B and Penny J B (1989). The functional anatomy of basal ganglia disorders. Trends Neurosci 12, 366 -375

Almazan G (2001). Pharmacological and functional characterization of muscarinic receptor subtypes in developing oligodendrocytes. J Neurochem 77, 1396-1406

Angelidis C E, Lazaridis I and Pagoulatos G N (1991). Constitutive expression of heat-shock protein70 in mammalian cells confers thermo-resistance. Eur J Biochem 199, 35-39

Arnt J and Skarsfeldt T (1998) Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. Neuropsychopharmacology. 18, 63-101

Augood S J, Herbison A E and Emson P C (1995). Localization of GAT-1 GABA transporter mRNA in rat striatum: cellular co-expression with GAD67 mRNA, immunoreactivity, and parvalbumin mRNA. J. Neurosci. 151, 865-874

Augood S J, Waldvogel H J, Munkle M C, Faull R L M and Emson P C (1999). Localization of calcium-binding proteins and GABA Transporter (GAT-1) messenger RNA in the human subthalamic nucleus. Neuroscience 88, 521-534

Aziz T Z, Peggs M A, Sambrook M A and Crossman A R (1991). Lesion of the subthalamic nucleus for the alleviation of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced parkinsonism in the primate. Movement Disorders 6, 288-292

Azmitia E C and Segal M (1978). An autoradiographic analysis of the differential ascending projections of the dorsal and medium raphe nuclei in the rat. J Comp. Neurobiol. 179, 641-668

Baimbridge K G and Celio M R Rogers J H (1992). Calcium-binding proteins in the nervous system. Trends Neurosci. 15, 303-308

Baldessarini R J and Frankenberg F R (1991). Clozapine. A Novel Antipsychotic Agent. New England J Med. 324, 746-754

Balsara J J, Jadhav J H and Chandorkar (1979). Effect of drugs influencing central serotonergic mechanisms on haloperidol-induced catalepsy. Psychopharmacology 62, 67-69

Barker E L, Westphal R S, Schmidt D and Sanders-Bush E (1994) constitutively active 5-hydroxytryptamine2C receptors reveal novel inverse agonist activity of receptor ligands. J Biol Chem 269, 11687-11690

Barnes N Mand Sharp T (1999). A review of central 5-HT receptors and their function Neuropharmacology 38, 1063-1152

Benjamin I J, Horie S, Greenberg M L, Alpern R J and Williams R S (1992): Induction of stress proteins in cultured myogenic cells. Molecular signals for the activation of heat shock transcription factor during ischimia. J Clin Invest 89, 1685-1689

Bentivoglio M, van der Kooy D and Kuypers H G M (1979). The organisation of the efferent projections of the substantia nigra in the rat. A retrograde fluorescent double labeling study. Brain Res 174, 1-117

Beasley C M, Tollefson G, Tran P, Satterlee W, Sanger T,Hamilton S and The Olanzapine HGAD Study Group. Olanzapine versus placebo and haloperidol: Acute phase results of the north American double-blind Olanzapine Trial. Neuropsychopharmacology14, 111-123

Bel D E A and Guimaraes F S (2000). Sub-chronic inhibition of nitric-oxide synthesis modifies haloperidol-induced catalepsy and the number of NADPH-diaphorase neurons in mice. Psychopharmacology 147, 356-361

Bevan M D, Bolam J P and Crossman A (1994). Convergent synaptic input from the neostriatum and the subthalamus on to identified nigrothalamic neurons in the rat. Eur J Neurosci 6, 320-334

Bevan M D, Crossman A and Bolam J P (1994). Neuron projecting from the entopeduncular nucleus to the thalamus receive convergent synaptic inputs from the subthalamic nucleus and the neostriatum. Brain Res 659, 99-109

Bevan M D, Francis C and Bolam J P (1995). The glutamate-enriched cortical and thalamic input to neurons in the subthalamic nucleus of the rat: convergence with GABA-positive terminals. J Comp Neurol 361, 491-511

Bigliani V, Mulligan R S, Action P D, Ohlsen R I, Pike V W, Ell P J, Gacinovic S, Kerwin R W and Pilwski (2000). Striatal and temporal single photon emission tomography (SPET) study. Psycopharmacology 150, 132-140

Birdsall N J M and Hulme E C (1983) Muscarinic receptor subclasses. Trends Pharmacol Sci 4, 459-463

Boess F G and Martin I L (1994). Review: Molecular Biology of 5-HT Receptors. Neuropharmacology. 33, 275-317

Bonhaus D W, Weinhardt K K, Taylor M, Desouza A, Mcneeley P M, Szczepanski K, Fontana D J, Trinh J, Rocha C L, Dawson MW, Flippin L A and Eglen R M (1997). RS-102221: A novel high affinity and selective, $5HT_{2C}$ receptor antagonist. Neuropharmacology 36, 621-629

Brown L L, Makman M H, Wolfson L I, Dvorkin D, Warner C and Katzman R (1979). A direct role of dopamine in the rat subthalamic nucleus and adjacent intrapeduncular area. Science 206, 1416-1418.

Brunello N, Masotto C, Steardo L, Markstein R and Racagni G (1995). New insights into the biology of schizophrenia through the mechanism of action of clozapine. Neuropsychopharmacology 13, 177-213

Brustowicz L M, Hodgkinson K A, Chow E W C, Honer W G and Bassett A S (2000). Location of a major vulnerability locus for mammalian schizophrenia on chromosome 1 q 21- q 22. Science 288, 678-682

Burstein E S, Spalding T A, and Brann M R (1997). Pharmacology of muscarinic receptor subtypes constitutively activated by G proteins. Mol Pharmacol 51, 312-319

Bymaster F P, Calligaro D O, Falcone J F, Marsh R D, Moore N A, Tye N C, Seeman P and Wong D T (1996). Radio-receptor binding profile of the Atypical antipsychotic olanzapine. Neuropsychopharmacology 14, 87-96

Bymaster F P, Rasmussen K, Calligaro D O, Nelson D L, DeLapp N W, Wong D T and Moor N A (1997): In vitro and in vivo biochemistry of olanzapine: a novel, atypical antipsychotic drugs. J Clin Psychiatr 58, 28-36

Bymaster F P, Shannon H E, Rasmussen T, DeLapp N W, Ward J S, Calligaro D O, Mitch C H, Whitesitt C, Ludvigsen T S, Shcardown M, Swedberg M, Rasmussen T,

Olesen P B, Jeppesen L, Sauerberg P and Jensen F (1999). Potential role of muscarinic receptors in schizophrenia. Life Scie. 64, 527-534

Bymaster F P, Shannon H E, Calligaro D O, K, DeLapp N W, Ward J S, Calligaro D O, Mitch C H, Whitesitt C, Ludvigsen T S, Sheardown M, Swedberg M, Rasmussen T, Caillard O, Moreno H, Schwaller B, Llano,I, Celio M R and Marty A (2000). Role of the calcium-binding protein parvalbumin in short-term synaptic plasticity. Proc Naional Acad Sci 97, 13375-13377

Calderon S F, Sanberg P R and Norman A B (1988). Quinolinic acid lesions of rat striatum abolish $D_1$ and $D_2$ dopamine receptor-mediated catalepsy. Brain Res 450, 403-407

Campbell G A, Eckardt M J and Weight F F (1985). Dopaminergic mechanism in subthalamic nucleus of rat: Analysis using horseradish peroxidase and microionophoresis. Brain Res 333, 261-270

Carlsson A and Lindqvist M (1963). Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol Toxicol 20, 140-144

Carlsson A, Lipton M A, Di Mascio A and Killman K F (eds) (1978). Mechanism of action of Neuroleptic drugs. Psycopharmacology. A generation of progress. Raven Press, New York, 1057-1070

Carlsson M and Carlsson A (1990). Interaction between glutamatergic and monoaminergic systems within the basal ganglia-implications for schizophrenia and Parkinson's disease. Trends Neurosci 13, 272-276

Carta A R and Grefen C R (1999). Lack of role for the $D_3$ receptor in clozapine induction of C-Fos demonstrated in $D_3$ dopamine receptor-deficient mice. Neuroscience 90, 1021-1029

Casey D E (1996). Behavioral effects of sertindole, risperidone, clozapine and haloperidol in Cebus monkeys. Psycopharmacology, 124, 134-140

Casey D E (1997). The relationship of pharmacology to side effects. J Clin Psychiatr, 58, 55-62

Celio M R (1990). Calbindin D-28k and parvalbumin in the rat nervous system. Neuroscience 35, 375-475

Celio M R and Heizmann C W (1981). Calcium-binding binding protein parvalbumin as a neuronal marker. Nature 293, 300-302

Chen J, Paredes W and Gardner E L (1991). Chronic treatment with Clozapine selectively decreases basal dopamine release in Nucleus-Accumbens but not in caudate putamen as measured by in vivo brain microdialysis-Further evidence for depolarisation block. Neurosci Lett. 122, 127-131

Coffin V L, McHuch D, Chopkin R E and Barnett A (1992). SCH 39166, a potential antipsychotic drug, does not evoke movement disorders in cebus monkeys. Neurochem Int 20, 141S-145S

Curran T, Gordon M B, Rubino K L and Sambucetti L C. Oncogene 2 , 79-84

Costall B and Naylor R J (1974). On catalepsy and catatonia and the predictability of the catalepsy test for neuroleptic activity. Psychopharmacology 34, 233-241

Costall B and Naylor R J (1975). Detection of the neuroleptic properties of Clozapine, Sulpiride and Thioridazine. Psycopharmacology (Berl.) 43, 69-74

Curzon G and Kennett G A (1990). m-CPP: a tool for studying behavioral responses associated with $5HT_{1C}$ receptors. Trends Pharmacol Sci. 11, 181-182

Dalman C, Thomas H V, David A S, Gentz J, Lewis G and Allebeck P (2001). Signs of asphyxia at birth and risk of schizophrenia: Population-based case-control study. Br J Psychiatr 179, 403-408

Dawson T M. Gehlert D R McCabe R T, Barnett A and Wamsley J K (1988). D-1 dopamine receptors in the rat brain: A quantitative autoradiographic analysis. J Neurosci 8, 2352-2365

Delay J and Deniker P (1952). Le treatment des psychoses par une method neuroleptique derive'e dc I'hibernotherapie. CR 50e Congress des alienistes et neurol de langue Fse 495-502

Delfs J M, Anegawa N J, and Chesselet M F (1995). Glutate decarboxylase messenger RNA in rat pallidum: comparison of the effects of haloperidol, clozapine and combined haloperidol-scopolamine treatments. Neuroscience 66, 67-80

DeLong M R, Crutcher M D and Georgopoulos A P (1985). Primate globus pallidus and subthalamic nucleus: functional organisation. J Neurophysiol 53, 530-543

DeLong M R (1990). Primate models of movement disorders of basal ganglia origin. Trends Neurosci. 13, 281- 285

Di Chiara G, Morelli M, Porceddu M L and Gessa G L (1979). Role of thalamic γ-aminobutyrate in motor functions:Catalepsy and ipsiversive turning after intrathalamic muscimol. Neuroscience 4, 1453-1465

Di Giovanni G, De Deurwaerdere P, Mascio M, Di Matteo V, Esposito E and Spampinato U (1999). Selective blockade of serotonin-2C/2B receptors enhances mesolimbic and mesostriatal dopaminergic function: A combined in vivo electrophysiological and microdialysis study. Neuroscience 91, 587-597

Di Matteo V, Di Giovanni G, Di Mascio M and Espotio E (1999) SB242084, a selective serotonin$_{2C}$ receptor antagonist, increases dopaminergic transmission in the mesolimbic system. Neuropharmacology 38, 1195-1205

Done D J, Crow T J, Johnston E C and Sacker A (1994). Childhood antecedent of Schizophrenia and affective illness: Social adjustment at age 7 and 11. Br Med J 309, 699-703

Dragunow M, Butterworth N, Waldvogel H, Faull R L M and Nicholson L F B (1995). Prolonged expression of Fos-related antigens, Jun B and TrkB in dopamine-denervated striatal neurons. Mol Brain Res. 30, 393-396

Dunstan R, Broekkamp C L and Lloyd K G (1981). Involvement of caudate nucleus, amygdala or reticular formation in neuroleptic and narcotic catalepsy. Pharmacol Biochem Behav 14, 169- 174

Eberle-Wang K, Lucki I and Chesselet M F (1996). A role for the subthalamic nucleus in 5-HT$_{2C}$ -induced oral dyskinesia. Neuroscience 72, 117-128

Eberle-Wang K, Mikeladze K, Uryu K and Chesselet M F (1997). Pattern of the Seronin$_{2C}$ Receptor Messenger RNA in the Basal Ganglia of Adult Rats. J Comp Neurol 384: 233-247

Eglen R M, Choppin A and Watson N (2001). Therapeutic opportunities from muscarinic receptor research. Trends Pharmacol Sci 22, 409-414

Elliot P J, Walsh D M, Close S P, Higgins G A and Hayes A G (1990). Behavioral effects of serotonin agonists and antagonists in the rat and marmoset. Neuropharmacology 29,949-956

Ennis C, Kemp J D and Cox B (1981). Characterisation of inhibitory 5- hydroxytryptamine receptors that modulate dopamine release in the striatum. J Neurochem 36, 1515-1520

Erlander M G, Tillakaratner N J K, Feldblum S, Patel N and Tobin A J (1991). Two genes encode distinct glutamate decarboxylases. Neuron, 7 91-100

Eve D J, Nisbet A P, Kingsbury A E, Hewson E L, Daniel S E, Lees A J, Marsden C D and Foster O J F (1998). Basal ganglia neuronal nitric oxide synthase mRNA expression in Parkinson's disease. Mol Brain Res 63, 62-71

Farde L, Nordstrom A L, Wiesel F A, Pauli S, Halldin C and Sedvall G (1992). Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine: relation to extrapyramidal side effects. Arch Gen Psychiatr 49, 538-544

Felder C C et al., (2000). Therapeutic opportunities for muscarinic receptors in the central nervous system. J Med Chem 43, 4333-4353

Forbes I T, Ham P, Booth D, Martin R, Thompson M, Baxter G S, Blackburn T P, Glen A, Kennett G A and Wood MD (1995). 5-methyl-l-(3-pyridycarbamoyl)-2,3-dihydropyrrolol[2,3-f] indole: a selective $5HT_{2B}$ / $5HT_{2C}$ receptor antagonist with improved potency, selectivity and oral activity. J. Med. Chem. 38, 2524-2530

Fox S H, Moser B and Brotchie, J M (1998). Behavioral effects of 5-HT2c receptor antagonism in the substantia nigra zona reticulata of the 6-hydroxydopamine-lesioned rat model of Parkinson's disease. Exp. Neurol. 151, 35-49

Fujimoto K and Kita H (1993). Response characteristics of subthalamic neurons to the stimulation of the sensorimotor cortex in the rat. Brain Res 609, 185-192

Garver D L, Schlemmer R F, Mass J W and Davis J M (1975) A schizophreniform behavioral psychosis mediated by dopamine. Am J Psychiatr. 132, 33-38

Gerfen C R (1992). The neostriatal mosaic: multiple levels of compartmental organisation in the basal ganglia. Annu Rev Neurosci 15, 285-320

Gerfen C R, Engber T M, Mathan L C, Susel Z, Chase T N, Monsma F J and Sibley R S (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striato-pallidal neurons. Science 250, 1429-1432

Gerfen C R, Keefe K A and Gauda E B (1995). D1 and D2 dopamine receptor function in the striatum; coactivation of D1 and D2 dopamine receptor receptors on separate populations of neurons results in potentiated immediate early gene responses in D1-containing neurons. J Neurosci 15, 8167-8176

Gershanik O, Heikkila R E and Duvoisin R C (1983). Behavioural correlations of dopamine receptor activation. Neurology 33, 1489-1492

Gessa G L, Devoto P, Diana M, Flore G, Melis M and Pistis M (2000). Dissociation of haloperidol, clozapine and olanzapine effects on electrical activity of mesocortical dopamine neurons and dopamine release in the prefrontal cortex. Neuropsychopharmacology 22, 642-649

Glazer W M (2000). Extrapyramidal Side Effects, Tardive Dyskinesia, and the Concept of Atypicality. J Clin Psychiatr, 61 (suppl 3) 16-21

Gonzalez M F, Siraishi K, Hisanaga K, Sagar S M, Mandabach M and Sharp F R (1989) Heat shock proteins as markers of neuronal injury. Mol Brain Res. 6, 93-100

Grace A A (1991). Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 41, 1-24

Greenberg M E and Ziff E B (1984). Stimulation of 3T3 cells induces transcription of c-fos proto-oncogene. Nature 311, 433-438

Guidotti A, Auta J, Davis J M, Gerevini Valeria D G, Dwivedi Y, Grayson D R, Impagnatiello F, Pandey G, Pesold C, Sharma R, Uzunov D and Costa E (2000). Decrease in Reelin and Glutamic Acid Decarboxylase 67 (GAD67) expression in Schizophrenia and Bipolar Disorder: A Postmortem Brain Study. Arch Gen Psychiatr 57, 1061-1069

Hafner H, An D H, Behrens W F, Gattaz M, Hambrecht M, Loffer W, Maurer P and Munk-Jorgenson P, Nowotny B, and Reicher-Rossler A, Stein A (1998). Causes and consequences of the gender difference in age at on set of schizophrenia. Schizophr. Bull 24, 99-113

Hassani O K, Mouroux M and Feger J (1996). Increased subthalamic neuronal activity after nigral dopaminergic lesion independent of disinhibition via the globus pallidus. Neuroscience 72, 105-115

Heimar L, Zahm D S and Alheid GF (1995). Basal Ganglia, in The Rat Nervous System, 2nd edition, edited by Paxinos G, School of psychopathology, the university of New South Wales, Sydney, Australia 579-628

Hendrick J P and Hart F U (1993). .Molecular chaperone functions of heat-shock proteins. Annu Rev Biochem 62, 349-384

Henderson J M, Annett L E, Ryan L J, Chiang W, Hidaka S, Torres E M and Dunnett (1999). Subthalamic nucleus lesions induce deficits as well as benefits in the hemiparkinsonian rat. Euro J Neurosci 11, 2749-2757

Herdegen T and Leah J D (1995). Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/AFT proteins. Brain Res Rev 28, 370-4

Heyser C J, Rajachandran L, Spear E N and Spear L P (1994). Responsiveness to cocaine challenge in adult rats following prenatal exposure to cocaine. Pharmacology 116, 45-55

Hicks P B (1990). The effect of serotonergic agents on haloperidol-induced catalepsy. Life Sci. 47, 1609-1615

Hontanilla B, Parent A, Heras S D L and Gimenez-Amaya J M (1998). Distribution of calbindin D-28k and parvalbumin neurons and fibers in the rat basal ganglia. Brain Res Bull 47, 107-116

Hontanilla B, Parent A and Gimenez-Amaya J M (1997). Parvalbumin and calbindin D-28k in the entopeduncular nucleus, subthalamic nucleus, and substantia nigra of rat as revealed by double-immunohistochemical methods. Synapse 25, 359-367

Hornykiewicz O (1973). Parkinsons disease: from brain homogenate to treatment. Fed Proc. 32, 183-190

Hutson P H, Barton C L, Jay M P, Lurton F, Burkamp R, Clarkson L and Bristow J (2000). Activation of mesolimbic dopamine function by 5-HT2c/2B receptor antagonists: neurochemical and behavioral studies. Neuropharmacology 39, 2318-2328

Jansson PA (1967). The pharmacology of haloperidol. Int J Neuropsychiatr 3, 10-18.

Jansson P (1970). The butyrophenone story. In Ayd F J, Blackwell B (eds), Discoveries in biological psychiatry. Lippicott, Philadelphia, 165-179

Jardemark K E, Al J, Ninan I P E and Wang R Y (2001). Biphasic Modulation of NMDA-Induced Responses in Pyramidal Cells of the Prefrontal Cortex by Y-931, a Potential Atypical Antipsychotic Drug. Synapse 41, 294-300

Jarskog L F, Gilmore J H, Selinger E S and Lieberman J A (2000). Cortical BCL-2 protein expression and apoptic regulation in schizophrenia. Biol Psychiatr 48, 641-651

Jaskiw G E, Collins K A, Pehek E A and Yamamoto B K (2001). Tyrosine augments acute clozapine but not haloperidol-induced dopamine release in the medial prefrontal cortex of rat: An in vivo microdialysis study. Neuropsychopharmacology 25, 149-156

Jian M, Staines W A, Iadarola M J and Robertson G S (1993). Destruction of nigrostriatal pathway increases Fos-like immunoreactivity predominantly in the striato-pallidal neurons. Mol. Brain Res. 19, 156-160

Johnstone E C, Crow T J, Frith C D, Husband J and Kreel L (1976). Cerebral ventricular size and cognitive impairment in chronic schizophrenia. Lancet 2, 924-926

Jones P, (1997). The early origins of schizophrenia. Br. Med. Bull. 51, 135-155

Jones P, Rogers B and Murray R (1994). Child development risk factors for adult Schizophrenia in British 1946 birth cohort. Lancet 344, 1398-1402

Kaichi Y, Noaka R, Hagino Y and Watanabe M (2001). Dopamine receptor binding by $D_3$ agonist 7-OH-DPAT (7-hydroxy-dipropylaminotetralin) and antipsychotic drugs measured ex vivo by quantitative Autoradiography. Can J Physiol Pharmacol 78, 7-11

Kalivas P W and Nakamura M (1999). Neural systems for behavioral activation and reward. Curr Opin Neurobiol 19, 223-227

Kalkman H O, Neumann V, Nozulak J and Triclebank M D (1998). Cataleptogenic effect of subtype selective 5-HT receptor antagonists in the rat. Eur J Pharmacol 343, 201-207

Kalyanchuk L E and Beck C H M (1992). Behavioral analysis of diazepam-induced memory deficits: evidence for sedation-like effects. Psychopharmacol 106, 297-302

Kamme F, Campbell K and Wielock T (1995). Biphasic expression of the fos and jun families of transcription factors following transient forebrain ischaemia in the rat. Effect of hypothermia. Eur J Neurosci. 7, 2007-2016

Kapur S, Zipursky R and Remington G (1999). Clinical and theoretical implications of $5HT_2$ and $D_2$ receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. Am J Psychiatr 156, 286-293

Kapur S, Zipursky R, Jones C, Remington G and Houle S (2000). Relationship between dopamine $D_2$ occupancy, clinical response, and side effects: A double-blind PET study of first-episode schizophrenia. Am J Psychiatr 157, 514 -520

Kapur S, Zipursky R, Jones C, Shammi C S, Remington G and Seeman P (2000b). A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D2 receptor occupancy. Arch Gen Psychiatr 57, 553-559

Kapur S and Seeman P (2001). Does fast dissociation from the dopamine $D_2$ receptors explain atypical antipsychotic action- a new hypothesis. Am J Psychiatr 158, 360-369

Kay G G and Harris A G (1999), Loratadine: a non-sedating antihistamine. Review of its effects on cognition, psychomotor performance, mood and sedation. Clin Exp Allergy 29, 147-150

Kennett G A, Whitton P, Shah K and Curzon G (1989). Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-$HT_{1C}$ receptor antagonists. Eur J Pharmacol 164: 445-454

Kennett G A (1993). 5-HT1C receptors and their therapeutic relevance. Curr Opin Invest Drugs 2, 317-362

Kennett G A, Wood A G, Grewal S, Forbes I T, Gadre A and Blackburn TP (1994). In vivo properties of SB 200646A, a 5-$HT_{2C/2B}$ receptor antagonist. Br J Pharmacol 111, 797-802

Kennett G A, Wood A G, Bright F, Cilia J, Piper D C, Gager T, Thomas D, Baxter G S, Forbes I T,Ham P and Blackburn T P (1996). In vitro and in vivo profile of SB 206,533, a potent 5-$HT_{2C}$/5-$HT_{2B}$ receptor antagonist, with anxiolytic-like properties. Br J Pharmacol, 117, 427-434

Kennett G A, Wood A G, Bright F, Trail B, Riley G and Holland V (1997). SB 242084, a selective and brain penetrant 5-$HT_{2c}$ receptor antagonist. Neuropharmacology 36, 609-620

Kinon B J and Lieberman J A (1996). Mechanisms of action of atypical antipsychotic drugs: a critical analysis. Psychopharmacology 124, 2-34

Kitai S T and Deniau J M (1981). Cortical inputs to the subthalamus: intracellular analysis. Brain Res 214, 411-415

Kita H and Kitai S T (1994). The morphology of globus pallidus projection neurons in the rat: an intracellular staining study. Brain Res. 636, 308-319

Klein D F and Davis J M (1969). Diagnosis and drug treatment of psychiatric disorders. Williams and Wilkins, Baltimore.

Kostowski W, Gumulka W and Czlonkowski (1972). Reduced cataleptogenic effects of some neuroleptics in rats with lesioned midbrain raphe and treated with *p*-chlorophenylalamine. Short communications. Brain Res 48, 443-446

Lawrie S M and Abukmeil S S (1998). Brain abnormality in schizophrenia. A systemic and quantitative review of volumetric magnetic resonance imaging studies. Br. J Psychiatr 172, 110-120

Lee M A, Thompson P A and Meltzer H Y (1994). Effects of Clozapine on cognitive function in schizophrenia. J Clin Psychiatr 55, 82-87

Leslie R A, Moorman J M, Coulson A and Grahame-Smith D G (1993). Serotonin 2/1C receptor activation causes a localised expression of the immediate early gene c-fos in the rat brain: evidence for involvement of dorsal raphe nucleus projection fibers. Neuroscience 53 , 457-463

Leufkens (2001). Antipsychotic-induced extrapyramidal syndromes. Eur J Clin Pharmacol 57, 327-331

Levy R, Hazrati L N, Herrero M T, Vila M, Hassani O K, Mouroux M, Ruberg M, Asensi H, Agid Y, Féger J, Obeso J A, Parent A and Hirsch E C (1997). Re-evaluation of the functional anatomy of the basal ganglia in normal and Parkinsonian state. Neuroscience 76, 335-343

Lewi P J, Heykants J J, Allewijn F T, Dony J G and Janssen P A (1970) Distribution and metabolism of neuroleptic drugs.I. Pharmacokinetics of haloperidol. Arzneimittelforschung 20, 943-948

Lewis D A (1997). Development of the prefrontal cortex during adolescence: insights into vulnerable neural circuits in schizophrenia. Neuropsychopharmacology 16, 385-398

Lewis D A (2000). Is there a neuropathology of schizophrenia ? The Neuroscientist 6, 208-218

Lewis D A and Lieberman J A (2000). Catching up on Schizophrenia. Neuron, 28, 325-334

Lipton J W, Ling Z, Vu T Q, Robie H C, Mangan K P, Weese-Mayer D and Carvey P M (1999). Parental cocaine exposure reduces glial cell line-derived neurotrophic factor (GDNF) in the striatum and the carotid body of the rat: implications for DA neurodevelopment. Developmental Brain Research 118, 231-235

Li X M, Perry K W, Wong D T and Bymaster F P (1998). Olanzapine increases in vivo dopamine and norepinephrine release in rat prefrontal cortex, nucleus accumbens and striatum. Psychopharmacology 136, 153-161

Lieberman J A, Safferman A Z, Pollack S, Szymanski S, Johns C, Howard A, Kronig M, Bookstein P and Kane J M (1994). Clinical effects of clozapine in Chronic-Schizophrenia – Response to treatment and Predictors of Outcome. Am J Psychiatr 151, 1744-1752.

Lillehoj E P, Ford G M, Bachmann S, Schroder J, Torry EF and Yolken RH (2000). Serum antibodies reactive with non-human primate retroviruses identified in acute onset schizophrenia. J Neurovirology 6 (6), 492-497

Lindquist S (1986). The heat-shock response. Ann Rev Biochem 55, 1151-1191

Lipton J W, Ling Z, Vu T Q, Robie H C, Mangan K P, Weese-Mayer D E and Carvey P M (1999). Prenatal cocaine exposure reduces glial cell line-derived neurotrophic factor (GDNF) in the striatum and the carotid body of the rat: implications for DA neurodevelopment. Dev Brain Res 118, 231-235

Lucas G, Bonhomme N, De Deurwaerdere P, Le Moal M and Spampinato U (1997). 8-OH-DPAT, a 5-$HT_{1A}$ agonist and ritanserin, a 5-$HT_{2A/C}$ antagonist, reverse haloperidol-induced catalepsy in rats independently of striatal dopamine release. Psychopharmacology 131, 57-63

MacGibbon, G.A., P.A.Lawlor, R. Bravo and Dragunow M. (1994). Clozapine and haloperidol produce a differential pattern of immediate early gene expression in rat

caudate-putamen, nucleus accumbens, lateral septum and islands of Calleja. Mol Brain Res 23, 21-32

Marcelis M, Van Os J, Sham P, Jones P, Gilvarry C, Cannon M, Mckenzie K, and Murray R (1998). Obstetric complications and familial morbid risk of Psychiatric disorder. Am J Med Genetics 81, 29-36

Marshall J F, Ruskin D N and LaHoste G J (1998). Dopaminergic regulation of immediate early gene expression in the basal ganglia. Advances in pharmacology 42, 678-681

Marsden C D and Obeso J A (1994) The functions of the basal ganglia and paradox of stereotaxic surgery in Parkinson's disease. Brain 117, 877-897

Massa, S M, Longo FM, Zuo J, Wang J, Chen and Sharp, F.R. (1995). Cloning of Rat grp75, an hsp 70-Family Member, and Its Expression in Normal and Ischemic Brain. J Neurosci Res 40, 807-819

Martin D L and Rimvall K (1993). Regulation of γ-aminobutyric acid synthesis in the brain. J Neurochem 60, 395-407

Martres M P, Boutenet M L, Sales N, Sokoloff P and Schwartz J C (1985). Widespread distribution of brain dopamine receptors evidenced with [$^{125}$I]- iodosulpiride, a highly selective ligand. Science 228, 752-755

Matteo V D, Giovanni G D, Mascio M D and Esposito E (1999). SB 242084, a selective serotonin$_{2c}$ receptor antagonist, increases dopaminergic transmission in the mesolimbic system. Neuropharmacology 38, 1195-1205

Mc Neil, T.F. (1991). Obstetric complications in schizophrenic parents. Schizophr Res 5, 89-101

Meltzer , H. Y, Matsubara, S. and Lee, M. A. (1989). Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin$_2$ pKi values. J Pharmacol. Exp Ther 251, 238-246

Meltzer , H. Y and Nas, J F (1991) Effects of antipsychotic drugs on serotonin receptors. Pharmacol Rev 43, 587-604

Meltzer, H. Y. (1995). Role of serotonin in the action of atypical antipsychotic drugs. Clin Neurosci 40, 885-887

Meltzer, H. Y. (1996). Pre-clinical pharmacology of atypical antipsychotic drugs: a selective review. Br J Psychiatr 168, 23-31

Meltzer HY and McGurk SR (1999). The effect of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. Schizophr Bull 25, 233-255

Miller R J and Hiley C R (1974) Anti-muscarinic properties of neuroleptics and drug induced Parkinsonism. Nature (Lond) 248, 596-597

Millar R J, Horn A S and Iversen L L (1974). The action of neuroleptic drugs on dopamine-stimulated adenosine cyclic 3, 5-monophosphate productions in rat neostriatum and limbic forebrain. Mol Pharmacol 10, 759-766

Millbrandt J (1987). A nerve growth factor-induced gene encodes a possible transcription regulatory factor. Science 238, 797-799

Mink J W and Thach W T (1993). Basal ganglia intrinsic circuits and their role in behavior. Curr Opin Neurobiol 3, 950-957

Miquel V, Wu D C, and Przedborski (2001). Engineered modeling and the secrets of Parkinson's disease. Trends Neurosci 24, S49-S55

Morelli M and Chiara G (1985). Catalepsy induced by SCH 23390 in rats. Euro J Pharmacol 117, 179-185

Morimoto R I (1993a). Chaperoning the nascent polypeptide chain. Curr. Opin. Biol. 3, 101- 102

Morimoto R I (1993b) Cells in stress: transcriptional activation of heat shock genes. Science 259, 1409-1410

Morimoto T, Fujimura M, Kuriyama M, Shoji K, Hasimoto K, Yasumatu H, Tanaka H, Takehara S and Yamagami K (2000). Y931, a novel antipsychotic with potential property to ameliorate NMDA receptor- hypofunction (NRH). I. Receptorial and pharmacological profile in comparison with clozapine and haloperidol. Drug discovery laboratories, Welfide Corporation, Iruma. Saitama, Japan. Soc Neurosci Abstr 26, 276

Morimoto T, Hashimoto K, Yasumatsu H, Tanaka H, Fujimura M, Kuriyama M, Kimura K, Takehara S, and Yamagami K (2002). Neuropharmacological Profile of a Novel Potential Atypical Antipsychotic Drug Y-931 (8-fluoro-12-(4-methylpiperazin-1-yl-6H-[1]benzothieno[2,3-b][1,5]benzodiazepinemaleate). Neuropsychopharmacology 26, 456-467

Mouroux M and Féger J (1993). Evidence that the parafascicular projection to the subthalamic nucleus is glutamatergic. Neuro Report 4, 613-615

Moore N A, Tye N C, Axton M S and Risius F C (1992). The behavoural pharmacology of olanzapine, a novel 'atypical' antipsychotic agent. J Pharmacol Exp Ther 262, 545-551

Munkle M C, Waldvogel H J and Faull R.L.M (2000). The distribution of Calbindin, calretinin and parvalbumin immunoreactivity in the human thalamus. J. Chem Neuroanatomy 19, 155-173

Muramatsu M , Tamaki-Ohashi J , Ushki C , Araki H , Chaki S and Aihara H (1988). 5-HT sub 2 antagonists and minaprine block the 5-HT-induced inhibition of dopamine release from rat brain striatal slices. Eur J Pharmacol 153, 89-95

Murer M G, Dziewczapolski G, Salin P, Vila M, Tseng KY, Ruberg M, Rubinstein M, Kelly Y, Grandy D K, Low M J, Hirsch E, Raisman-Vozari R and Gershanik O (2000). The indirect basal ganglia pathway in dopamine $D_2$ receptor-deficient mice. Neuroscience 99, 643-650

Najlerahim A, Harrison P J, Barton A J L, Heffernan J and Pearson R C A (1990). Distribution of messenger RNAs encoding the enzymes glutaminase, aspartate aminotransferase and glutamic acid decarboxylase in rat brain. Mol Brain Res. 7, 317-333

Neal-Beliveau B S, Joyce J N and Luki R (1993). Serotonergic Involvement in Haloperidol-Induced Catalepsy. J Pharmacol Expt Therap. 265, 207-217

Nestler E J (1997). An emerging pathophysiology. Nature 385, 578-579

Nguyen T V, Kosofsky B E, Birnbaum R, Cohen B M and Hyman S E (1992). Differential expression of c-fos and zif268 in rat striatum after haloperidol, clozapine, and amphetamine. Proc. Natl. Sci. USA89, 4270-4274

Nisbet A P, Foster O J F, Kingsbury A, Lees A J and Marsden C D (1994). Nitric oxide synthase mRNA expression in human subthalamic nucleus, striatum and globus pallidus: implications for basal ganglia function. Mol Brain Res 22, 329-332

Nistch C, Scotti A, Sommacal A and Kalt G (1989). GABAergic hippocampal neurons resistant to ischimia-induced delayed neural death contain the calcium binding protein parvalbumin. Neurosci Lett 105, 263-268

Nowak T S (1990). Protein synthesis and heat shock response after ischaemia. Cerebrovasc Brain Metabol Rev 2, 345- 366

Nowak T J, Sborne O C and Suga S (1993). Stress protein and proto-oncogene expression as indicators of neuronal pathophysiology after ischaemia. Prog Brain Res 96, 195-208

Ögren S O, Håkan H, Christer K, Olle M and Sven-Erik S (1986). The selective dopamine $D_2$ receptor antagonist raclopride discriminates between dopamine-mediated motor functions. Psychopharmacology 90, 287-294

Ögren S O and Fuxe K (1988). $D_1$ and $D_2$ – receptor antagonist induce catalepsy via different efferent. Striatal pathways. Neurosci Lett 85 333-338

Olivier C, Herman M, Beat S, Llano I, Marco R C, and Alian M (2000). Role of the calcium-binding protein parvalbumin in short-term synaptic plasticity. Proc National Acca Sci 97, 13372-13377

O'Neill M F Heron-Maxwell C L and Shaw G(1999). 5-$HT_2$ receptor antagonism reduces hyperactivity induced by amphetamine, cocaine, and MK-801 but not D1 agonist C-APB. Pharmacol Biochem and Behaviour 63, 237- 243

Olesen P H, Jeppesen L, Sauerberg P and Fink-Jensen A (1999). Potential role of muscarinic receptors in Schizophrenia. Life sci. 64, 527-534

Onn S P, West A R and Grace A A (2000). Dopamine-mediated regulation of striatal neuronal and network interactions. Trends Neurosci 23, S48-S56

Ossowska K, Wedzony K and Wolfarth S (1984). The role of the GABA mechanisms of the globus pallidus in mediating catalepsy, stereotypy and locomotor activity. Pharmacol Biochem and Behaviour 21, 825-831

Palacios J M, Waeber C, Hoyer, D and Mengod G (1990). Distribution of serotonin receptors. The Neuropharmacology of serotonin. Annu N Y Acad Sci 600, 36-52

Parent A, Mackey A, Smith Y and Boucher R. (1983). The output organisation of the substantia nigra in primate as revealed by a retrograde double labeling method. Brain Res Bull 10, 529-538

Parent A and Lavoie B (1993). Dopaminergic innervation of the basalganglia in normal and parkinsonian monkeys. In CurrentConcepts in Parkinson's Disease Research (eds Schneider J. S and Gupta M), pp. 403-414. Hans Huber, Toronto

Parent A and Lavoie B (1995). Chemical anatomy of primate basal ganglia. Prog. Neurobiol. 46, 131-197

Parent A and Hazrati L N (1995). Functional anatomy of the basal ganglia I. The cortico-basal ganglia-thalamo-cortical loop. Brain Res Rev 20, 91-127

Parent A and Hazrati L N (1995). Functional anatomy of the basal ganglia-II. The place of STN and external pallidum in basal ganglia circuitry. Brain Res Rev 20, 128-154

Pasquletti Mori M, Castagna D, Cassano G B and Nardi I (1999). Distribution and cellular localization of the serotonin type 2C receptor messenger RNA in human brain. Neuroscience. 92, 601-611

Paxinos G and Watson C (1998), The Rat Brain in Stereotaxic Coordinates, 4 Th. Ed. San Diego: Academic Press.

Planas A M, Marc A, Soriano A E, Sanz O, Mmartin F and Ferrer I (1997). The heat shock stress response after brain lesions: Induction of 72 kDa heat shock protein (cell types involved, axonal transport, transcriptional regulation) and protein synthesis inhibition. Prog Neurobiol, 51, 607-636

Pompeiano M (1994). Distribution of the serotonin5-$HT_2$ receptor family mRNAs: comparison between 5-$HT_{2A}$ and 5HT $_{2C}$ receptors. Mol. Brain Res 23, 163-178

Poyurovsky M and Weizman A (1997). Serotonergic agents in the treatment of acute neuroleptic-induced akathisia: open-label study of buspirone and mianserin. Int Clin Psychopharmacol. 12, 263-268

Pulver A E (2000). Search for schizophrenia vulnerability genes. Biol. Psychiatr. 47, 221-230

Rajakumar N, Elisevich K and Flumerfelt B A. (1994) Parvalbumin-containing GABAergic neurons in the basal ganglia output system of the rat. J Comp Neurol 350, 324-336

Martin D L and Rimvall K (1993). Regulation of γ-aminobutyric acid synthesis in the brain. J Neurochem 60,395-407

Rattray M and Priestley J V (1993) Differential expression of GABA transporter-1 messenger RNA in subpopulations of GABA neurons. Neurosci Lett 156, 163-166

Readler T J, Knable M B and Weinberger D R (2000). Schizophrenia as a developmental disorder of the cerebral cortex. Curr Opinion Neurobiol 8, 157-161

Reavill A, Kettle V, Holland G Rily and T P Blackburn (1999). Attenuation of haloperidol-induced catalepsy by a 5-$HT_{2C}$ receptor antagonist. Br J Pharmacol 126, 572-574

Reus V I (1997). Olanzapine: a novel atypical neuroleptic agent (Commentary). Lancet, 349, 1264-1265

Reynolds G P, Beassley C L (2001). GABAergic neuronal subtypes in the human frontal cortex – development and deficits in schizophrenia. J Chem Neuroanatomy 22, 95-100

Robertson G S and Fibiger H C (1996). Effects of olanzapine on regional C-Fos expression in rat forebrain. Neuropsychopharmacology 14, 105-110

Robertson G S, Matsumura H and Fibiger H C (1994) Induction pattern of Fos like immunoreactivity in the forebrain as predictors of atypical antipsychotic activity. J Pharmacol Exp Ther 271, 1058-1066

Robledo P and Féger J (1990). Excitatory influence of rat subthalamic nucleus to substantia nigra pars reticulata and the pallidal complex: electrophysiological data. Brain Res, 518, 47-54

Ruskin D N and Marshall J F (1995). D1 dopamine receptors influence Fos immunoreactivity in the globus pallidus and subthalamic nucleus of intact and nigrostriatal-lesioned rats. Brain Res 703, 156-164

Sakai K, Gao X, Hashimoto, K and Tamminga, CA. (2001). Traditional and new antipsychotic drugs differentially alter neurotransmission marker in basal ganglia-thalamocortical neural pathways. Synapse 39, 152-160

Sakai K, Gao X-M, Hashimoto T and Tamminga C A (2001). Traditional and New Antipsychotic Drugs Differentially Alter Neurotransmission Markers in Basal Ganglia-Thalamocortical Neural Pathways. Synapse 39, 152-160

Salin P, Dziewczapolski G, Gershanik O, Nieoullon A, and Raisman-Vozari R (1997). Differential regional effects of long-term L-DOPA treatment on preproenkephalin and preprotachykinin gene expression in the striatum of 6-hydroxydopamine-lesioned rats. Mol Brain Res, 47, 311-321

Sanberg R P, Faulks I J and Fibiger H C (1980). Experiential influences on catalepsy. Psychopharmacology 69, 225-226

Sanberg R P, Bunsey M, Giordano M and Norman A B (1988). The Catalepsy Test: Its Ups and Downs. Behav Neurosci 102, 748-759

Schroeder J A and Schneider J S (2001). Alterations in expression of messenger RNAs encoding two isoforms of glutamic acid decarboxylase in the globus pallidus and entopeduncular nucleus in animals symptomatic for and recovered from experimental Parkinsonism. Brain Res, 888, 180-183

Schillevoort A de Boer, Herings R M C, Roos R A C, Jansen P.AF, H G.M Scordo, Spina E, Romeo P, Dahl M L, Bertilsson L, Johansson I and Sjoqvist F (2000). CYP2D6 genotype and antipsychotic-induced extrapyramidal side effects in schizophrenic patients. Eur J Clin Pharmacol. 56, 679-683

Schotte A, Janssen P F M, Gommeren W, Luyten W H M L, VanGompel P, Lesage A S, DeLoore K and Leysen J E (1996). Risperidone compared with new and reference antipsychotic drugs in vitro and in vivo receptor binding. Psycopharmacology. 124, 57-73

Schultz W (1997). Dopamine neurons and their role in reward mechanisms. Curr Opin Neurobiol 7, 191-197

Scordo M G, Spina E, Romeo P, Dahl M-L, Bertilsson L, Johansson I and Sjoyist F (2000). CY2D6 genotype and antipsychotic-induced extrapyramidal side effects in schizophrenic patients. Eur J Clin Pharmacol 26, 679-683

Sebens J B, Koch T, Gert J Ter Host and J Korf (1998). Olanzapine-induced fos expression in the rat forebrain; cross-tolerance with haloperidol and clozapine. Eur J Pharmacol. 353, 13-21

Seeman P, Lee T, Chau-Wong M and Wong K (1976). Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 61, 717-719

Seeman P, Corbett R and Van Tol H H (1997). Atypical neuroleptics have low affinity for DA $D_2$ receptors, or are selective for $D_4$ receptors. Neuropsychopharmacology 16, 93-110

Sen G and Bose K C (1931). Rauwolfia serpentina, a new Indian drug for insanity and high blood pressure. Indian Med World 2, 194-201

Sharp F R, Jasper P, Hall J, Noble L and Sagar S M (1991). MK-801 and ketamine induce heat shock protein HSP72 in injured neurons in posterior cingulate and retrosplenial cortex. Annu Neurol 30, 801-809

Shinde U A, Mehta A A and Goyal R K (2000). Nitric Oxide: A molecule of the millennium. Indian J Expt Biol 38, 201-210

Siddiqui and Siddiqui (1931-1939). In Medicinal Chemistry (1964): Edited by G DeStevens: Psychopharmacological Agents, edited by Maxwell Gordon. 1

Smith Y, Bevan M D; Shink E and Bolam (1998). Microcircuitry of the direct and indirect pathways of the basal ganglia. Neuroscience 86, 353-387

Soghomonian J J and Martin D L (1998). Two isoforms of glutamate decarboxylase: Why? Trends Pharmacol Sci 19, 500-505

Sokoloff P, Levvesque D, Martres M P, Lannfelt L, Diaz G and Pilon C (1992a). The dopamine D3 receptor as a key target for antipsychotics. Clin Neuropharmacol 15, 456A-457A

Sokoloff P, Martres M P, Giros B, Bouthenet M L and Schwartz J K (1992b): The third dopamine receptor ($D_3$) as a novel target for antipsychotics. Biochem Pharmacol 43, 659-666

Staines W A and Fibiger H C (1984). Collateral projections of neurons of the rat globus pallidus to the striatum and substantia nigra. Exp. Brain Res 56, 217-220

Stanwood G D, Washington R A, Shumsky J S, and Levitt P (2001). Parental cocaine exposure produces consistent developmental alteration in dopamine-rich regions of the cerebral cortex. Neuroscience 106, 5-14

Stefulj S, Jermej B, Cicin-Sain L, Rinner I and Schauenstein K (2000). RNA expression of serotonin receptors in cells of the rat. Brain Behav Immun 14, 219- 224

Sugimoto T and Hattori T(1984). Organisation and efferent projections of nucleus tegmenti pedunculopontinus pers compacta with special reference to its cholinergic aspects. Neuroscience 11, 931-946

Sulway M R, Bore G A, Creascy H, Dent O F, Jorm A F, Kos S C and Tennant C C (1996). Are malnutrition and stress risk factors for accelerated cognitive decline? A prisoner of war study. Neurology 46, 650-655

Sumeier D J, Song W J and Yan Z (1996). Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. J Neurosci 16, 6579-6591

Szymanski S, Lieberman J A, Alvir J M , Mayerhoff D, Lobel A, Geisler S, Chakos M, Koreen A, Jody D and Kane J (1995). Gender differences in onset of illness, treatment response, course, and biologic indexes in first-episode schizophrenic patients. Am J Psychiatr 152, 698- 703

Tandon R, Milner K, Jibson M D (1999). Antipsychotics from theory to practice: integrating clinical and basic data. J Clin Psychiatr 60, 21-28

Thomas H V, Dalman C, David A S, Gentz J, Lewis G and Allebeck P (2001). Obstetric complications and risk of schizophrenia: Effect of gender, age at diagnosis and maternal history of psychosis. Br J Psychiatr 179, 409-414

Toru M and Takashima M (1985). Haloperidol in large doses reduces the cataleptic response and increases noradrenaline metabolism in the brain of the rat. Neuropharmacology 24, 231-236

Van der Kooy D and Hattori T (1980). Single subthalamic neurons project to both the globus pallidus and the substantia nigra in the rat. J Comp Neurol 192, 751-790

Van Horn J D and McManus I C (1992). Ventricular enlargement in schizophrenia. A meta-analysis of studies of the ventricle: brain ratio (VBR) [see comments]. Br J Psychiatr 161, 714-715

Van Prag H M, Korf J and Dols L C W (1976). Clozapine versus Perphenazine: The value of the Biochemical Mode of Action of Neuroleptics in predicting their therapeutic Activity. Br J Psychiatr 129, 547-55

Vezina P, Blanc G, Glowinski J and Tassin J-P (1994). Blockade of $D_1$ dopamine receptors in the medial prefrontal cortex produces delayed effects on pre and postsynaptic indices of dopamine function in the nucleus accumbens. Synapse 16, 104-112

Vila M, Herrero M T, Levy R, Faucheux B, Ruberg M, Guillen J, Luquin M R, Guridi J, Javoy-Agid F, Agid Y, Obeso J A and Hirsch E C (1997). Consequences of nigrostriatal denervation on the gamma-aminobutyric acidic neurons of substantia nigra pars reticulata and superior colliculus in parkinsonian syndromes. Neurology 46, 802-809

Vila M, Périer C, Féger J, Yelnik J, Faucheux B, Ruberg M, Raisman-Vozari R, Agid Y and Hirsch E C (2000). Evolution of changes in neural activity in the subthalamic nucleus of rats with unilateral lesion of the substantia nigra assessed by metabolic and electrophysiological measurements. Eur J Nerosci 12, 337-344

Visser S J de, Post J van der, Pieters M S M, Cohen and Gerven J M A (2001). Biomarkers for the effects of antipsychotic drugs in healthy volunteers. Br Clin Pharmacol 51, 119-132

Wadenberg M-L G (1992). Antagonism by 8-OH-DPAT, but not ritanserin, of catalepsy induced by SCH 23390 in the rat. J Neural Trans 89, 49-59

Wadenberg M-L G (1996). Serotonergic mechanisms in neuroleptic-induced catalepsy in the rat. Neurosci Biobehav Rev 20, 325-33

Wadenberg M-L G, Hicks P B, Young K A and Richter J T (1997). Further evidence for a role of the 5-HT2 receptor in the mediation of antipsychotic activity. Schizophr Res 24, 83

Wadenberg M-L G and Hicks P.B. (1999). The conditioned avoidance response test re-evaluated: is it a sensitive test for the detection of potentially atypical antipsychotics? Neurosci Biobehav Rev 23, 851-862

Wadenberg M-L G, Soliman A, VanderSpek S C and Kapur S (2001). Dopamine $D_2$ receptor occupancy is a common mechanism underlying animal models of antipsychotics and their clinical effects. Neuropsychopharmacology 25, 633-641

Waldmeier P C and Delini-St A A (1979). Serotonin-dopamine interactions in the nigrostriatal system. Eur J Pharmacol 55, 363-373

Weinberger D R (1987). Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatr 44, 660-669

White F J, Bednarz L M, Wachtel S R, Hjorth S and Brooderson R J (1988). Is stimulation of both $D_1$ and $D_2$ receptors necessary for the expression of dopamine-mediated behaviours? Pharmcol Biochem and Behav 30, 189-193

Williams M N and Faull R L M (1985). The striatonigral projection and nigrotectal neurons in the rat. A correlated light and electron microscopic study demonstrating a monosynaptic striatal input to identified nigrotectal neurons using a combined degeneration and horseradish peroxidase procedure. Neuroscience 14, 991-1010

Wisden W and Morris B J (1994). In situ hybridization with Synthetic oligonucleotide probes. Academic Press Limited. Biological Techniques Series

Wolf W A and Schutz L J (1997). The serotonin 5-$HT_{2C}$ receptor is a prominent serotonin receptor in basal ganglia: evidence from functional studies on serotonin-mediated phosphinositide hydrolysis. J Neurochem 69, 1449-1458

Wood M D, Reavill C, Trail B, Wilson A, Stean T, Kennett G A, Lightowler S, Blackburn T P, Thomas D, Gager T L, Riley G, Holland V, Bromidge S M, Forbes I T and Middlemiss DN (2001). SB-243213; a selective HT2c receptor inverse agonist with improved anxiolytic profile: lack of tolerance and withdrawal anxiety. Neuropharmacology 41 186- 199

Young K L, Smith A D, Levey A I and Bolam J P (1996). Synaptic connections between spiny neurons of the direct and indirect pathways in the neostriatum of the rat: evidence from dopamine receptor and neuropeptide immunostaining. Eur J Neurosci 8, 861-869

Zhang W and Bymaster F P (1999). The in vivo effects of olanzapine and other antipsychotic agents on receptor occupancy and antagonism of dopamine $D_1$, $D_2$, $D_3$, $5HT_{2A}$ and muscarinic receptors. Psychopharmacology 141, 267-278