TYPICAL AND ATYPICAL ANTIPSYCHOTICS: ROLE OF THE NORADRENERGIC SYSTEM IN THE TREATMENT OF SCHIZOPHRENIA

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SUMMARY

Currently two broad categories of drugs known as “typical antipsychotics” and “atypical antipsychotics” are used in the treatment of schizophrenia. The typical (e.g. haloperidol) were the first to be used and are known to be effective in treating the positive symptoms of the disease. The atypicals (e.g. clozapine, olanzapine) are the newer drugs and are genetally more effective in treating the negative symptoms.

The exact cause of better efficacy of the atypical drugs is not precisely known. In my research work, I have focused on the role of the noradrenergic system. I have investigated the effect of antipsychotics on immediate early gene (IEG) and tyrosine hydroxylase (TH) expression in the medial prefrontal cortex (mPFC) and locus coeruleus (LC) in rat brain. In addition I validated an animal model of schizophrenia by conducting prepulse inhibition (PPI) and latent inhibition (LI) studies in the genetically modified “*chakragati (ckr)”* mice. Effects of antipsychotic drugs and noradrenergic drugs on the PPI in these mice were also studied. In the last part of the thesis, experiments were conducted to study the effect of antipsychotic drugs and noradrenergic drugs on the PPI and water maze performance in an N-methyl-D-aspartic acid (NMDA) antagonist induced model of schizophrenia.

The study involving the IEG expression changes demonstrated that atypical and typical antipsychotics differ qualitatively in their effects on IEG and TH expression in the mPFC and LC. In particular, the atypical antipsychotics, risperidone and clozapine, produce greater increases in TH expression in the LC and mPFC than the typical antipsychotic,

haloperidol. I also charted effects of different olanzapine doses and treatment durations on IEG and TH protein expression in the mPFC and LC of the rat. There are immediate as well as delayed dose-dependent effects of olanzapine on the patterns of expression. Future investigation of how changes in IEG and TH expression correlate with each other in the mPFC and to prefrontal cortical dependent behaviours is required.

It was found that the *ckr* mice have disrupted LI and PPI. These effects were attributed to sensorimotor gating defects. I further showed that atypical antipsychotics were more successful in reversing the PPI defects than the typical antipsychotics. Over all the *ckr* mice has given indication that in future it could serve as a useful animal model of schizophrenia.

The experiments with adrenergic drugs, both in *ckr* mice as well as rats, show an additive effect of the alpha1 antagonist, prazosin, and atypical antipsychotics in reversing PPI deficits. In spatial memory tests in rats, there seemed to be an additive effect of the alpha2 antagonist, idazoxan, with the atypical antipsychotics, in improving the water maze performance.

Starting from IEG expression to behavior testing in animals, a role for adrenergic system is visible in the patho-psysiology as well as treatment of schizophrenia. The additive effects of adrenergic drugs to the atypical antipsychotic drugs is encouraging and has the potential to develop into a novel therapeutic regime.

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ABBREVIATIONS

1. **ANOVA**: Analysis of variance
2. **AP-1**: Activator protein – 1
3. **DAB**: Diamino benzidine
4. **ECT**: Electroconvulsive therapy
5. **EPS**: Extra pyramidal side-effects
6. **HRP**: Horse radish peroxidase
7. **IEG**: Immediate Early Gene
8. **LC**: Locus Coeruleus
9. **LI**: Latent Inhibition
10. **mPFC**: Medial Prefrontal Cortex .
11. **NMDA**: N-methyl-D-aspartate
12. **NPE**: Non preexposed
13. **PBS**: Phosphate buffered saline
14. **PCP**: Phencyclidine
15. **PE**: Preexposed
16. **PPI**: Prepulse Inhibition.
17. **SD**: Sprague Dawley
18. **TH**: Tyrosine hydroxylase

INTRODUCTION

1.1 SCHIZOPHRENIA

1.1.1 Disease and History

Schizophrenic disorders as defined by the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, are mental disorders which impair functioning and are characterized by psychotic symptoms involving disturbances of thought, perception, feeling and behavior (American Psychiatric Association, 1994). Six specific criteria for the diagnosis of schizophrenic disorders include (i) psychotic symptoms of delusions, hallucinations, formal thought disorder; (ii) deterioration from a previous level of functioning; (iii) chronicity of the disorder for at least 6 month; (iv) a tendency toward onset before the age of 45; (v) symptoms not due to mood (affective) disorders; and (vi) symptoms not due to organic mental disorder or mental retardation (American Psychiatric Association, 1994).

The incidence of schizophrenic disorders varies depending on the breadth of criteria used. Using a relatively narrow concept of the disorder, studies of European and Asian populations show a lifetime prevalence of 0.2% to almost 1% (McGrath et al, 2004). Although paranoid schizophrenia typically has a later onset, most schizophrenia

manifests itself in the late adolescence or early adult life (American Psychiatric Association, 1994). A higher prevalence in lower socioeconomic classes is also observed, which has been mainly attributed to social disorganization and consequent stresses. There is evidence to suggest that this association arises partly because some patients in a pre-psychotic phase drift down the social scale (Goodman et al, 1983).

Many patients who develop schizophrenia show pre-morbid personality traits such as hypersensitivity, a shyness, unsociability, lack of affect and paranoid attitudes (Erkwoh et al, 2003). Recently, a two syndrome hypothesis of schizophrenia suggests that there are 2 main types of schizophrenia (Huppert and Smith, 2005). Type 1, or positive schizophrenia, is characterized by acute onset, good pre-morbid adjustment, prominent positive symptoms, good response to drug therapy, and hyperdopaminergic transmission. Type 2, or the negative syndrome, is characterized by insidious onset, poor pre-morbid adjustment, prominent negative symptoms, cognitive impairment, structural brain abnormalities, and poor response to treatment (Huppert and Smith, 2005).

1.1.2 Signs and Symptoms

Thought disorder: Clear, goal-oriented thinking becomes a challenge, as shown in a diffuseness and incoherence of speech. Sudden and incomprehensible changes of subject and flaws in reasoning occur due to distractions of thought processes. Some schizophrenics may claim that their thoughts are being broadcast or shared with others; delusional interpretations of these experiences lead to the belief that their minds are being controlled by external agencies.

Emotional (affective) changes: Blunting and inappropriateness of affect are the most characteristic emotional changes noted in schizophrenic patients however, this can be difficult to evaluate as their assessment is subjective and unreliable. Withdrawal from external reality and failure to coordinate internal drives are frequent findings.

Perceptual disorder: Auditory hallucinations are the most common but hallucinations of sight, touch, smell and taste may occur. Specifically the hallucinations of a running commentary on the patient's actions or of voices talking about the patient, strongly suggest schizophrenia. Poverty of speech is commonly reported, and ritualistic behavior associated with magical thinking often occurs.

Delusions: Delusions of persecution are frequent, as are those involving hypochondriacal or religious ideas, jealousy, grandeur and sexual identity. Delusional interpretations of strange thoughts and conversations or that they are under the control of an external agency may seem illuminating to the patient but is incomprehensible to others.

Catatonic signs: Movement disturbances range from hyperactivity and excitement to marked retardation and ever stupor. In some cases, posturing may occur by which the patient may take up a bizarre position for prolonged periods. Extreme negativism or automatic obedience is sometimes seen. Mannerisms such as a facial contortion or overemphasis of normal movements are more common. There may be abnormalities of psychomotor activities; eg, rocking, pacing, peculiar motor responses and even immobility.

Violent behavior: In acute schizophrenic states and relapses, minor aggression and threats of violence are common but dangerous behavior when the patient obeys commanding voices is uncommon. The risk of suicide is increased in all stages of schizophrenia. Ten percent of schizophrenic patients commit suicide.

1.1.3 Treatment of Schizophrenia

Current treatment of schizophrenia relies primarily on somatic drug therapy. But the pharmacological treatment of schizophrenia did not begin, however, until approximately a century ago. Before this, all kinds of mental illnesses were thought to be related to religious causes. People suffering from mental illnesses were hidden away, institutionalized or executed. The first major advancement was made when it was discovered that exogenous opiates had mood altering effects. It was realized that there could be a pathological cause and an appropriate drug treatment for schizophrenia. In the present day other treatments of schizophrenia consist of sedatives, electroconvulsive therapy (ECT), artificially induced comas and frontal lobotomies. Though each treatment had its benefits, the side effects and the complications associated with them, often outweighed any therapeutic results (Kaplan and Sadock, 1995).

In 1963 Carleson and Lindquist noted the impact of antipsychotic medication on dopamine metabolism (Kaplan and Sadock, 1995). Since then there has been a tremendous increase in the pharmacological knowledge pertaining to the treatment of schizophrenia. Chlorpromazine was discovered in 1979. This revolutionized psychiatric treatment. It was originally thought of as a sedative for the cattle, but soon its value in

treating schizophrenia in humans was realized (Hemmings and Hemmings, 1978). Currently two broad categories of antipsychotics are used in the treatment of schizophrenia. They have been called typical and atypical antipsychotics.

The typical antipsychotics (previously known as neuroleptics) were the first group of antipsychotics to be used routinely in the treatment of schizophrenia. They primarily acted on the brain dopaminergic pathways and showed very little regional specificity. They are very effective in controlling the positive symptoms of the disease. These drugs also have many serious side effects. These range from neuroleptic malignant syndrome to extrapyramidal side effects (EPS) or even tardive dyskinesia. They are still commonly used in many countries but over the last few years they have lost their pre-eminent position to the drugs belonging to the atypical group. Common example of the typical antipsychotic is haloperidol.

Atypical antipsychotics are the newer group of drugs. The commonly used atypical antipsychotics include clozapine (the prototype drug for this category), risperidone and olanzapine. Atypical antipsychotic drugs not only produce less extrapyramidal side effects than typical antipsychotics but also show better efficacy against the negative and cognitive symptoms of schizophrenia (Kasper and Resinger, 2003; Meltzer and McGurk, 1999; Tandon and Jibson, 2003). In contrast, the typical antipsychotics may even exacerbate the negative and cognitive symptoms of schizophrenia (Kasper and Resinger, 2003; King, 1998; Markowitz et al., 1999). Many studies have shown that most patients show improved symptomatology on being treated by atypical drug clozapine. Of the

neuroleptic resistant subjects 79 % showed superior clinical results with clozapine (Baldessarini and Frankenburg, 1991).

1.2 THEORIES OF SCHIZOPHRENIA

Schizophrenia is generally considered a biochemical disorder of the brain. This has prompted many researchers to study the various chemicals and the chemical pathways involved in the brain function. Following are the prominent pathways, which have been studied:

1.2.1 The Dopaminergic Pathway

The first model for this pathway was the dopamine hyperactivity hypothesis. This states that the hyperactivity of the brain's dopaminergic systems is directly responsible for the symptoms of schizophrenia (Hemmings and Hemmings, 1978). Supporting this theory are observations related to the actions of the drugs that antagonize the activity of dopaminergic systems.

1.2.2 The Serotonergic Pathway

Serotonin's (5-hydroxytryptamine, 5-HT) role in schizophrenia was first recognized in the 1950s when people noted its similarity to psychosis produced by lysergic acid diethyl amide (LSD). LSD was known to produce symptoms of psychosis and it did so by acting on the serotonin receptors. A "hyper serotonin" hypothesis was formed because of this.

Further studies, which probed brain-behavior relationships, neurotransmitter systems, drug mechanisms and post mortem studies, provided more evidence of serotonin's involvement in schizophrenia. Atypical antipsychotics combine very weakly with dopaminergic receptors and it was suggested that they act on serotonin receptors. When atypical antipsychotics were combined with 5-HT2 antagonists, there occurred substantial relief in the negative symptoms of the patients (Kaplan and Sadock, 1995). This further highlighted the involvement of serotonin in schizophrenic pathophysiology. In addition a dopamine-serotonin interaction was also proposed whereby increased levels of serotonin in the prefrontal cortex caused the dopamine levels to fall. These reduced dopamine levels, which could cause the negative symptoms, further lead to increased dopamine levels in secondary dopaminergic systems. This increase is probably responsible for the positive symptoms. Serotonin's exact role is still not clear though.

1.2.3 The Glutamatergic Pathways

Deficiency in glutamatergic pathways was first suggested by Kim et al, 1980. They observed a reduced concentration of glutamate in the cerebrospinal fluid of a group of schizophrenic patients, compared to control subjects. A primary deficit in cortico-striatal glutaminergic neurotransmission was suggested, which led to an increase in nigrostriatal dopaminergic output. Subsequent studies failed to support this hypothesis though (Gattaz et al, 1982; Korpi et al, 1987a; Perry, 1982; Prieto-Rincon et al, 1991; Toru et al, 1988). Actions of a NMDA glutamate antagonist Phencyclidine (PCP), have also helped in implicating glutamate in the pathogenesis of schizophrenia. Domino (1980), and Javitt (1987) showed that PCP psychosis was a good drug model of schizophrenia. This is considered to be due to reduced NMDA glutamate receptor function. Although if we

follow this hypothesis, then the drugs which enhance glutamate activity should improve psychotic symptoms. In reality studies have not supported this (Cascella et al, 1994; Costa et al, 1990, Javitt et al, 1994).

1.2.4 The GABAergic Pathways

This pathway was first suggested by Roberts (1972). It was mentioned that a combination of reduced GABAergic function as well as an imbalance between dopaminergic-GABAergic system is responsible for schizophrenia. There were few studies which showed a reduction of GABA in certain parts of the brain (Perry et al, 1979; Spokes et al, 1980) but some other refuted these findings (Cross et al, 1979; Korpi et al, 1987b).

1.2.5 The Noradrenergic Pathway

The major part of this thesis is dedicated to investigation of this pathway. All the experiments were conducted to investigate role of the noradrenergic pathway in the actions of drugs used to treat schizophrenia. Noradrenaline is a catecholamine found in high concentrations throughout the nervous system. The system consists of a positive and negative feedback circuits, which affects the concentration levels of both noradrenaline as well as dopamine. The neurotransmitter acts on alpha and beta-receptors. The involvement of this pathway was proposed as early as 1971 (Stein and Wise, 1971; Hartman, 1976; and Hornykiewicz, 1982, 1986). Later on Van Kammen et al (1991) too provided evidence in support of this theory when they linked noradrenergic system to negative symptoms of schizophrenia.

1.3 ANIMAL RESEARCH IN SCHIZOPHRENIA

1.3.1 Animal Models of Schizophrenia

Over the past few decades various scientific teams have tried different animal models of schizophrenia. They vary from drug-induced models, to gene manipulation models. These models have been shown to mimic various symptoms of schizophrenia ranging from cognitive function like working memory to motor function like hyperactivity. A model of working memory deficits associated with schizophrenia can be seen by administering the non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist drug phencyclidine (PCP) in animals (Jentsch et al 1997). This same drug has also been shown to produce psychomimetic effects in humans (Buuse, 2005; Krystal et al 1994; Malhotra et al 1996). A hyperactivity model can be produced by administering amphetamine to the experimental animals (Creese and Iverson, 1975; Geyer and Markou, 1995; Buuse, 2005).

It is difficult to produce all the symptoms of schizophrenia in a single model, but a few features can be produced consistently, which have good validity. In these experiments it is important that the test has “Construct Validity”. This refers to the similarity in the underlying mechanisms that are involved in a particular behavior, although these behaviors may be expressed in a different way in humans and experimental animals (Buuse, 2005). As discussed by Kilts (2001), there are two very prominent areas of studies regarding the symptoms of schizophrenia. They are related to deficits in the sensory processing of stimuli, which show up as stimulus filtering and attentional

impairment. Prepulse inhibition (PPI) and latent inhibition (LI) are two behavioural phenomenas, which are related to the sensory inhibition processes that are impaired in schizophrenia. Tests like the Morris water maze can be used to assess the spatial memory changes in rodent models of schizophrenia, created by administering drugs like PCP or dizocilipine (MK-801). These are discussed in greater detail in the following paragraphs.

Another area where a lot of work has been done recently is the creation of genetic-based models of schizophrenia. Rapid improvement in technology has aided this a great deal. These genetic manipulations can be broadly classified as "reverse" genetic approach or "forward" genetic approach (Kilts, 2001). The "reverse" methods have been discussed in detail in an article by Tarantino & Bucan (2000). The method involves creating a genetic change in the animal and then proceeding to look at the behavioral changes caused by that genetic manipulation. The "forward" approach entails looking out for schizophrenic characteristics in an animal first and then proceeding to analyze it's genetic make up.

This thesis investigated the genetically modified "*Chakragati* (ckr)" mice. These mice were serendipitously created as a result of a transgenic insertional mutation (Torres et al., 2004). A 24-kb genomic fragment containing the mouse Ren-2^{d} rennin gene was microinjected into BCF (c57BL/$10Ros^{pd}$ x C3H/HeRos) fertilized oocytes (Ratty et al., 1990). Genetic and physical analysis of this insertion revealed that 2.5 copies of the transgene, comprising 65-70 kb, had integrated, duplicated and inverted portions of a particular locus within chromosome 16 of the mouse genome. The apparent loss-of-function of the endogenous gene produced a mice that in homozygous condition, exhibited abnormal circling behaviour phenotype. Futher it was found that this

phenotypic behaviour could be corrected by atypical antipsychotics clozapine and olanzapine. The increased motor activity of these mice was similar to that observed in wild type animals treated with dizocilipine, a NMDA receptor antagonist that produces behaviour resembling the positive symptoms of schizophrenia.

1.3.2 Prepulse Inhibition (PPI)

PPI is a sensory-motor gating phenomenon, which is found to be in deficit in schizophrenic patients (Kumari et al, 1999) as well as animal models (Mansbach and Geyer, 1989; Keith et al, 1991; Bakshi et al, 1994; Wedzony et al, 1994; Swerdlow et al, 1996) of schizophrenia. In the experimental set up, when a loud sound stimulus presented to an animal, is preceeded by a weak sound, the startle response to the loud sound gets attenuated. Although the pre-stimulus weak sound is not able to elicit a response on its own, it does activate the inhibitory mechanisms, which gate further stimulation until the processing of the prepulse has been completed. Over all there is a disrupted processing and reduced impact of the pulse, and hence the PPI effect (Kumari and Sharma, 2002).

In experimental set ups in laboratories acoustic stimuli are used both as pulse (strong sound) as well as the prepulse (weak sound). Usually the animal is placed on a transducer platform, which is located in a sound attenuated box. Sound stimuli are provided to the animal and it's startle reaction is captured by the transducer platform. This reaction is then quantified with the help of computer software. This test is considered to have good predictive, face and construct validity for schizophrenia (Braff and Geyer, 1990).

Research has showed that there are various ways in which deficits in PPI can be induced in animals. Although these animals are not the perfect model of schizophrenia as a whole, they do serve as good model of the sensori-motor gating problems associated with schizophrenia (Geyer and Markou, 2001). PPI deficit can be produced by stimulation of D_2 dopamine receptors, with amphetamine or apomorphine (Davis, 1988); by activation of serotonergic system, produced by 5-HT releasers or direct agonists at multiple serotonin receptors (Kehne et al, 1996; Padich et al, 1996); by blocking of N-methyl-D-aspartate (NMDA) receptors, produced by drugs like phencyclidine (PCP) (Johansson et al. 1995); or by developmental manipulations of the animals, like rearing in isolation (Varty et al. 1999).

1.3.3 Latent Inhibition

LI is one of the behavioral phenomena seen in schizophrenic patients. If a person or an animal is provided with a repeated stimulus, which is not followed by any significant consequence, there is observed, retarded conditioning to that stimulus. This is in comparison to a new stimulus to which the person or organism had not been exposed before. Normal LI is modulated by attentional processes. These processes are not working properly in the case of schizophrenics and hence we see disrupted LI in these subjects (Lubow, 2005). LI is seen to quantify an organism's ability to ignore irrelevant stimuli (Lubow, 1973; Lubow, 1989; Lubow and Gewirtz, 1995). It helps the organism concentrate more on newer inputs rather than the older unimportant one (Lubow, 2005). Various studies have linked latent inhibition to schizophrenia (Braff and Geyer, 1990; Gray et al., 1991; Feldon and Weiner, 1992; Gray, 1998).

Validity of the relationship between LI and schizophrenia was shown when amphetamine, which produces positive symptoms of schizophrenia in normal subjects, decreased LI in rat studies (Ellinwood, 1967; Zahn et al. 1981). Similarly it has been shown that atypical antipsychotics like clozapine (Moran et al. 1996), olanzapine (Gosselin et al. 1996) and remoxipride (Trimble et al. 1997) produced the expected increase in LI or prevented the LI lowering effect of indirect dopamine agents (Moser et al. 2000; Weiner, 2000; Tzschentke, 2001).

1.3.4 Morris Water Maze

Cognitive impairment is seen in patients of schizophrenia. This is also seen in some animal models of schizophrenia. One test which is routinely employed to assess spatial memory is the Morris water maze. The open field water maze is an apparatus in which rodents are trained to escape from the water by swimming to a hidden platform. The location of this platform can only be identified using extra-mazal cues. The water maze task was introduced by Morris (1981) and colleagues as a spatial localization or navigation task. The task has been extensively used to study the neurobiological mechanisms that underlie spatial learning and memory, age associated changes in spatial navigation (Gage et al. 1984; Rapp et al. 1987; Pitsikas et al. 1990), and the ability of psychopharmacological agents (Sutherland et al. 1982; Hagan et al. 1983; McNaughton and Morris, 1987), lesions (Morris et al. 1982; Kolb et al. 1983) or gene mutations (Tsien et al. 1996; Crawley et al. 1997) to influence specific cognitive processes.

The water maze challenge tests a set of “cognitive” processes in the animal whereby the process involved in the storage and retrieval of spatial information interact with the

process involved in planning and navigational strategies. Performance in the water maze can be affected by lots of factors and these should be considered before comparing the results of any two experiments. These factors could be the sex of the animal, their strain, the dimensions of the pool which has been used for the experiment, the temperature of the water when the experiment were conducted and the particular training schedule which was followed during the study (Wenk, 1998). The results of the test can also vary due to the factors, which can effect the swim speed of the animal. These could include the body weight of the animal, it's muscle development, and its age. Brandeis et al (1989) made a detailed comment on the role of these factors on the water maze performance of the animals.

The water maze task is a labor-intensive task where the experimenter needs to be involved at all the times. As far as problems related to the experiment itself are concerned, there are two major areas of concern: first is the stress caused to the animal when it is immersed into the water. This stress may cause endocrinological changes in the animal as such might go on to interfere with the experimental results (Wenk, 1998). This problem can be solved by continued exposure to the pool. The second problem is related to the method by which the pool water is made opaque. If powered milk is used, then the pool needs to be cleared everyday otherwise there could be a bacterial contamination and odour which might arise very quickly in the pool water. If some coloring agent is used then it needs to be taken into account that it is not toxic to the animal (Wenk, 1998).

1.3.5 Immediate Early Genes (IEGs) Expression

Immediate early genes (IEGs) are genes whose induction is a primary response to an external stimulus. It is not secondary to other waves of gene expression. IEGs and their proteins are links through which external stimuli can alter the gene transcription process within a cell. A large number of IEGs have been identified. A few of them for example, c-fos, c-jun and egr-1 fall under the category of "transcription factors". These are DNA binding proteins having several related homologs (Hai and Curran, 1991; Nakabeppu et al, 1988). Most of them have the ability to form homodimers and heterodimers amongst themselves and then attach to promoters such as the activator protein (AP-1) consensus site (TGACTCA). Affinity of each of these complexes for the AP-1 site is different from the others (Hai and Curran, 1991; Kovary and Bravo, 1991; Ryseck and Bravo, 1991). Also these complexes sometimes undergo posttranslational modifications that further enhance their ability to affect the transcription process in a more diverse way (Barber and Verma, 1987; Ofir et al, 1990; Boyle et al, 1991).

Induction of expression of c-Fos and related Fos-like immediate early gene proteins has been considered a marker of neuronal activation (Sagar et al., 1988; Dragunow and Faull, 1989) and has been used to map the brain regions activated by antipsychotic drugs in numerous studies (Deutch and Duman, 1996; Fink-Jensen and Kristensen, 1994; Robertson et al., 1994; Robertson and Fibiger, 1992; Robertson and Fibiger, 1996).

Both atypical and typical antipsychotics induce expression of Fos-like immunoreactivity in the shell of the nucleus accumbens. In the dorsolateral striatum, while typical antipsychotics strongly induce Fos expression, atypical antipsychotics only weakly

induce expression of Fos-like immunoreactivity (Deutch and Duman, 1996; Fink-Jensen and Kristensen, 1994; Robertson et. al., 1994; Robertson and Fibiger, 1992; Robertson and Fibiger, 1996). The difference in the extent to which antipsychotics induce expression of Fos-like immunoreactivity in the shell of the nucleus accumbens and the dorsolateral striatum has been proposed as a measure of "atypicality" reflecting the likelihood that they will produce extrapyramidal side effects (Robertson et. al., 1994).

The prefrontal cortex is involved in working memory and executive function (Callicott et. al., 1999; Dalley et al., 2004; Goldman-Rakic, 1996; Robbins, 1996) and is well characterized as a site of abnormal brain function in schizophrenia (Bunney and Bunney, 2000; Callicott and Weinberger, 1999; Goldman-Rakic, 1999; Goldman-Rakic and Selemon, 1997; Weinberger et. al., 2001). Hence, because atypical antipsychotics, but not typical antipsychotics, readily induce expression of Fos-like immunoreactivity in the prefrontal cortex (Deutch and Duman, 1996; Robertson et al., 1994; Robertson and Fibiger, 1992; Robertson and Fibiger, 1996), it has been suggested that the induction of Fos-like immunoreactivity in the prefrontal cortex may correlate with the greater efficacy of atypical antipsychotics against the negative symptoms and cognitive dysfunction in schizophrenia (Deutch and Duman, 1996; Robertson et al., 1994; Robertson and Fibiger, 1992; Robertson and Fibiger, 1996; Ananth et. al., 2001)

1.4 NORADRENERGIC THEORY OF SCHIZOPHRENIA

1.4.1 Role of the Noradrenergic system in PPI

Studies have been conducted which point towards an involvement of the noradrenergic system in the occurrence of prepulse inhibition. Bakshi and Geyer (1997) showed that the PPI deficit caused in experimental animals by administering the psychomimetic drug phencyclidine (PCP), could be reversed by prazosin, an alpha-1 noradrenergic antagonist. In the same experiment they also tested the impact of alpha-2 antagonist RX821002 in reversing the effect of PCP. But the alpha-2 antagonist failed to show any effect. In a separate experiment Bakshi and Geyer (1999) showed that alpha-1 adrenergic receptors mediated the sensorimotor gating deficits produced by intracerebral dizocilipine administration in rats. In this experiment they administered quetiapine (a drug having strong alpha-1 affinity) and prazosin, 15 minutes prior to bilateral infusion of dizocilipine into either the dorsal hippocampus or amygdala. Both quetiapine and prazosin blocked the PPI deficit producing effect of dizocilipine. In 1998, Carasso et. al. showed that cirazoline, the alpha-1 adrenergic agonist disrupted PPI in rats and that this effect was reversed by prazosin and atypical antipsychotics. Disruption of PPI by cirazoline was also reported by Shilling et. Al. (2004). Another study done by Mishima et. al. (2004), reported that mutant mice lacking alpha-1d-adrenergic receptors showed lower levels of acoustic startle responses than the wild-type group at lower pulse intensities, although the acoustic prepulse inhibition was not impaired in the alpha-1d knockout mice. It was also reported that MK-801 (Dizocilipine) induced deficits of PPI were not observed in these

knockout mice. All these clearly suggest a role of the alpha-1 adrenergic receptors in PPI mechanism.

A few studies have also looked into the role of alpha-2 receptors in PPI. Sallinen et. al. (1998) worked in a genetically modified mouse in which they inactivated the gene encoding alpha-2C adrenergic receptor. The animal showed enhanced startle response as well as diminished PPI. In animals with tissue specific over expression of the alpha-2c receptors was associated with the opposite effects. Lahdesmaki et. al. (2004) also were involved in an experiment with genetically modified mice in which alpha-2A receptor was deleted. The paper suggests that the alpha-2 adrenoceptors regulate the excitability and transmitter release of brain monoaminergic neurons mainly as inhibitory presynaptic auto- and hetero-receptors. The knockout mice, when treated with D-amphetamine, showed increased startle responses and more pronounced disruption of PPI. The startle attenuation was not observed after administering the alpha-2 agonist dexmedetomide in the knockout mice as compared to the wild type. Shishkina et. al. (2004) also showed the involvement of the alpha-2 adrenoceptors in the process of PPI. In a recent article, Powell et. al. (2005) have shown that alpha-2 antagonist drugs like yohimbine and atipamezole decreases PPI in experimental animals, while the alpha-2 agonist, clonidine, showed an increase in PPI.

Overall these studies point towards an alpha-1 antagonistic or alpha-2 agonistic mechanism for increasing PPI, while the decrease in PPI has been suggested to be because of alpha-1 adrenoceptor antagonism or alpha-2 noradrenoceptor agonism.

1.4.2 Noradrenergic System and IEG Expression

As discussed previously, antipsychotic drugs lead to IEG expression changes in the brain. The induction of Fos-like immunoreactivity in the prefrontal cortex by atypical antipsychotics, clozapine and olanzapine, was blocked by the beta-adrenoceptor antagonist, propranolol (Ohashi et al., 2000). As atypical antipsychotics are not reported to exhibit beta agonist activity, this suggests that activation of the LC, the major source of noradrenergic innervation of the prefrontal cortex (Berridge and Waterhouse, 2003), and release of noradrenaline is instrumental in inducing this Fos-like activity in the mPFC.

Consistent with this hypothesis, acute administration of atypical antipsychotics has been shown to increase c-Fos and Fos-like immunoreactivity in the LC (Dawe et. al., 2001; Ohashi et. al., 2000), increase the firing rate of LC cells (Dawe et al., 2001; Nilsson et al., 2005; Ramirez and Wang, 1986; Souto et al., 1979), and release noradrenaline in the prefrontal cortex (Nutt et. al., 1997; Li et. al., 1998; Westerink et. al., 1998). Importantly however, while the acute activation of c-Fos expression in the prefrontal cortex appears to be unique to atypical antipsychotics and dependent upon beta-adrenoceptors, both typical and atypical antipsychotics can activate firing of the LC. Haloperidol also increases the firing rate and burst firing of LC cells (Dinan and Aston-Jones, 1984; Nilsson et. al., 2005), although arguably less so than clozapine (Nilsson et. al., 2005). Moreover, acute haloperidol also increases noradrenaline release in the prefrontal cortex (Westerink et. al., 1998), although perhaps to a lesser degree than risperidone and clozapine, and although it induces less Fos-like activation in the prefrontal cortex, the activation that they produce is beta-adrenoceptor sensitive (Ohashi et. al., 1998). These data based on acute administration of antipsychotics seem to imply that the difference in

effects of typical and atypical antipsychotics is quantitative rather than qualitative, which is not consistent with clinical findings. However, clinically antipsychotics are invariably administered chronically and the benefits of atypical antipsychotics against negative symptoms and cognitive dysfunction appear to be manifest later than their effects on positive symptoms and are seen most markedly after several weeks of treatment (Stahl, 2005).

It would be interesting to compare the effects of chronic treatment with typical and atypical antipsychotics on immunoreactivity to an antibody to c-Fos in the mPFC and LC. Other stimuli, such as stress and nicotine administration, that induce activation of the LC are reported to induce increases in expression of tyrosine hydroxylase (TH) the rate limiting enzyme in catecholamine synthesis (Kvetnansky and Sabban, 1998; Mitchell et. al., 1993; Sabban et. al., 2004; Serova et. al., 1999; Smith et. al., 1991; Zigmond et. al., 1974). Likewise, chronic treatment with high doses of olanzapine have been reported to increase TH expression in the LC (Ordway and Szebeni, 2004). TH expression may influence release of noradrenaline in the prefrontal cortex. It is not known whether chronic treatment with other antipsychotics similarly influences TH expression. Both AP-1 complex Fos family proteins and Egr-1 have been linked to induction of TH expression (Nakashima et. al., 2003; Papanikolaou and Sabban, 1999; Papanikolaou and Sabban, 2000). Therefore in the present study, we have incorporated the investigation of TH immunoreactivity and the expression of two Egr-family immediate early gene proteins.

1.4.3 Noradrenergic System and Performance in the Water Maze

Several studies have been performed to look into the role of the noradrenergic system and it's receptors in spatial navigational tasks. Usually these tasks are impaired in schizophrenic patients as well as schizophrenic animal models. The studies have implicated both the alpha-1 and alpha-2 adrenoceptors to certain extent. Suggestions have also been made about the involvenment of beta adrenoceptors. Bjorklund et. al. (1998; 1999; 2000) worked with genetically modified mice, which over expressed for alpha-2C receptors. These animals were found to be impaired in spatial water maze tests. Following treatment with alpha-2 antagonist drugs, like atipamezole, this impairment was fully reversed. Chopin et. al. (2002) used dexefaroxan, a potent and selective alpha-2 adrenoceptor antagonist to study it's effect on spatial memory processes in the Morris water maze tasks in rats. Dexefaroxan facilitated the spatial memory processes and ameliorated the age related memory deficits of 24 month old rats to a level that was compatible with that of adult animals. In a separate experiment Chopin et. al. (2004) again showed the protective effects of dexefaroxan against spatial memory deficit induced by cortical devascularization in the adult rat.

A few studies have also implicated the role of alpha-1 adrenoceptors. Puumala et. al. (1998) showed that administration of St-587 (a putative alpha-1 agonist) improved water maze navigation to a hidden platform in rats. In the same experiment they also showed that pre-training administration of St-587 ameliorated scopolamine induced impairment in the performance of rats. In a separate experiment Riekkinen et. al. (1997) also showed that treatment with St-587 facilitated acquisition of water maze spatial navigation in rats.

Spreng et. al. (2001) showed while working with alpha-1 adrenoceptor knockout mice, that these animals were unable to learn water maze task.

Interestingly enough a few studies have also looked into the role of beta adrenoceptors. Ji et. al. (2003) showed that DL-propranolol, the beta adrenergic antagonist causes a deficit in 48 hr memory for the spatial water maze task in rats, when administered 5 minutes post training. Over all they mention that beta-adrenoceptors are involved in regulating consolidation of spatial memory for the water maze. In a conflicting study Decker et. al. (1990) mention that pretraining administration of propranolol has no effect on the spatial learning in Morris water maze.

Thse data signify involvement of alpha-1 and alpha-2 adrenoceptors in spatial learning tasks of water maze. Alpha-2 antagonism or alpha-1 agonism seems to improve the process in rodents. Experiments done by Arnsten and her team on monkeys, show contrary results. Their studies suggest that alpha-2 agonism improves, while alpha-1 agonism impairs, spatial working memory in monkeys (Arnsten and Jentsch, 1997; Arnsten et. al., 1988). These contrary results in rodents and monkeys could be due to species difference and I need investigate whether my experiments with rats show results similar to rodents or to the monkey.

1.5 AIM OF THE THESIS

Typical and atypical antipsychotics play a major role in the treatment of schizophrenia. The atypical ones, for example clozapine and olanzapine, have shown better efficacy in treating the cognitive and negative symptoms of schizophrenic patients. The exact reason for this is not known. Since these drugs act on multiple neurotransmitter receptors, it is possible that actions at a combination of these receptors is the cause of this superiority of atypical antipsychotics. Over the years it has also been observed that the noradrenergic drugs tend to play an important role in schizophrenic charecteristics. It seemed very interesting to investigate the role of noradrenergic system and its interaction with the antipsychotic drugs.

This thesis analyzes the role of noradrenergic system in the superiority of atypical antipsychotics over typical ones. There are three parts to the thesis. They are as follows:

Part I: I have investigated the effect of chronic antipsychotic drug administration on the expression of IEG expression in LC and PFC. I have also investigated the expression of TH, the rate limiting enzyme in catecholamine synthesis. We studied if there was any up-regulation, i.e., an increase in IEG expression or down-regulation, i.e., a decrease in the expression levels of the IEGs.

Part II: I conducted baseline PPI and LI experiments on the transgenic *chakragati* mice so as to validate these tests in this putative animal model of schizophrenia. This was

followed by investigating the effects of antipsychotics and noradrenergic drugs on PPI in this mouse model.

Part III: I have investigated the effect of treatment with antipsychotics and noradrenergic drugs on PPI as well as spatial navigation performance tasks in rats. These animals were administered antipsychotics and noradrenergic drugs separately as well as simultaneously, so as to observe their independent effects and also their concomitant interactions.

SUMMARY OF AIMS :

1. To investigate the effects of chronic antipsychotic administration on IEG and TH expression in LC and PFC.

2. To investigate the *chakragati* mouse as a model of schizophrenic deficit in PPI and LI tests in *chakragati* mice and observe the effect of antipsychotic and noradrenergic drug treatments on their PPI.

3. To investigate the hypothesis that the noradrenergic system is involved in the effects of antipsychotics on PPI and spatial navigational memory in rats.

METHODOLOGY

2.1 IMMUNOSTAINING EXPERIMENTS

2.1.1 Comparison between typical and atypical antipsychotics:

Subjects

Adult male Sprague-Dawley rats (Laboratory Animals Centre, National University of Singapore) weighing 180-200 g at the start of treatment were used. Animals were group housed and maintained on a 12 h light / 12 h dark cycle (lights on 07:00-19:00 h) in a temperature-controlled (22 ºC) colony room with *ad libitum* access to food and water. All experiments were approved by the institutional animal ethics review board of the National University of Singapore and were conducted in accordance with the International Guiding Principles for Animal Research (Howard-Jones, 1985).

Drug Treatment

After one week of acclimatization in the colony room, the rats were randomly assigned to groups for chronic treatment with antipsychotic drugs or vehicle (n = 8 per group). The drugs haloperidol (Sigma), clozapine (Tocris) and risperidone (Sigma) were dissolved in distilled water acidified to pH 4.5 to 5 with acetic acid. Three groups were administered antipsychotic drugs once daily subcutaneously for 4 weeks: haloperidol (4 mg/kg/day), clozapine (10 mg/kg/day) and risperidone (1 mg/kg/day). The fourth group was

administered acidified saline (0.9% NaCl in distilled water acidified to pH 4.5 to 5 with acetic acid) once daily subcutaneously for 4 weeks. Injections were administered between 16:00 h and 17:00 h.

Perfusion and Tissue Processing

Between 16 and 18 hours after the final injection, the rats were anaesthetized with an overdose of sodium pentobarbital and fixed by transcardial perfusion with 0.9% saline followed by 4% paraformaldehyde in phosphate buffer (pH 7.4). The brains of the rats were recovered, divided into four coronal blocks of 5 mm each, and post-fixed for 2 to 3 days 4% paraformaldehyde in phosphate buffer (pH 7.4) before paraffin embedding with an automatic tissue processor (Leica TP1020, Leica Microsystems, Germany). The blocks were serially sectioned at 6 μm on a rotary microtome (Leitz 1512, Leica Microsystems, Germany) and mounted on slides.

Immunohistochemistry

Alternate serial sections through the mPFC and LC were immunostained with antibodies against c-Fos, Egr-1, Egr-2 and TH. For immunohistochemistry the sections were processed as previously described (Dawe et. al., 2001) with minor modifications. Briefly, the sections were dewaxed in xylene and rehydrated through an ethanol series to distilled water. The tissue was then quenched for endogenous peroxidase activity by treating it with 0.3% hydrogen peroxide. After washing the tissue thrice with distilled water, normal serum (from the species donating the secondary antibody) was added to block nonspecific background staining. This was followed by application of the primary antibody to the

sections. Sections were then left to incubate under appropriate conditions. For post incubation, the tissue was washed thrice with phosphate buffered saline (PBS) and treated with biotinylated secondary antibody. This was followed by three washes with PBS and application of an avidin-biotinylated HRP complex (rabbit ABC staining system, Santa Cruz Biotechnology, CA, USA). Again the tissue was washed three times with PBS. Immunoreactivity in the tissues was visualized using the diaminobenzidine (DAB) chromogen.

All the primary antibodies were rabbit polyclonal antibodies. The antibodies against the IEGs were from Santa Cruz Biotechnology, CA, USA. The antibody against TH was from Chemicon International, CA, USA. The primary antibodies were initially titrated from 1:50 to 1:1000 with incubations of 12 to 72 hours, both at room temperature and in a fridge at 4 ºC. The following incubation protocols were adopted: anti-c-Fos (1:100, 72 hours at room temperature), anti-Egr-1 (1:100, 48 hours at room temperature), anti-Egr-2 (1:50, 24 hours at 4 ºC) and anti-TH (1:150, 24 hours at room temperature).

Counting of labelled cells

Images of 400 x 400 μm areas were captured using a light microscope (BX51, Olympus, Japan) and a digital camera (Magnafire SP, Optronics, CA, USA). The prelimbic area of the mPFC was sampled at approximately 2.7 mm anterior to bregma. The LC was sampled at approximately 9.8 mm posterior to bregma. The LC was identified by histological landmarks, including juxtaposition to the large cells of the mesencephalic nucleus of the Vth nerve (Me5), and with reference to sequential sections immunostained

for TH. Immunopositive cells were counted only within the LC. Area of the LC sampled by the 400 x 400 μm box was measured. Immunopositive cells were counted according to a protocol adapted from procedures previously described (Dawe et. al., 2001). The immunoreactive nuclei in the region of interest were detected by binary segmentation to a fixed threshold and application of a binary dilation-erosion filter to remove artifacts (Image Pro Plus, Media Cybernetics Inc, MD, USA). The segmentation threshold was fixed for each antibody across all samples. For sections immunostained for sections immunostained for the immediate early genes, fixed object size and roundness filters were applied to select for immunoreactive nuclei. For sections immunostained with TH, the procedure was modified to count immunoreactive profiles of axonal and somatodendritic elements for quantification of immunoreactivity in the mPFC and LC. Four regions of interest were sampled bilaterally from two sections at least 72 μm apart in each brain and the mean number of immunoreactive nuclei or profiles per μm^2 was calculated. The data are expressed as the mean percentage change in the number of immunoreactive nuclei or profiles relative to the pooled mean count for vehicle-treated control group (mean ± std).

Statistics

Data were analyzed by one-way analysis of variance (ANOVA) for the effect of drug treatment followed by post-hoc Dunnet's tests against the acidified saline vehicle-treated control group and Tukey's honestly significant difference test between drug treatment groups. All tests were applied with a two-tailed significance criterion of $p < 0.05$.

2.1.2 Effect of different dosages and treatment durations of Olanzapine on IEG and TH expression

Subjects

Adult male Sprague-Dawley rats (180-200 g) were obtained from the Laboratory Animals Centre, National University of Singapore. They were group-housed with free access to food and water. A 12 h:12 h light:dark cycle was maintained. All experiments were approved by the institutional animal ethics review board of the National University of Singapore and were conducted in accordance with the International Guiding Principles for Animal Research (Howard-Jones, 1985).

Drugs

Olanzapine (Eli Lilly and Company, Indianapolis, IN, USA) was dissolved in distilled water acidified to pH 5.5 by application of 1 M HCl and adjusted back to pH 6.0 with 1 M NaOH. Saline (0.9% NaCl in distilled water) was acidified to pH 6.0 by application of 1 M HCl.

Implantation of osmotic pumps

Rats were anaesthetized with sevoflurane (8 % for induction and 3 to 4 % for maintenance) and osmotic minipumps (Alzet Model 2ML2 or 2ML4, Durect Corporation, CA, USA) were implanted subcutaneously. The rats (n = 64) received either 2, 4, 8, or 15 mg/kg/day of olanzapine for durations of either 4 hours, 1 week, 2 weeks, or 4 weeks (n = 4 rats for each dose at each treatment duration).

Perfusion fixation and tissue processing

This was done as described earlier in the section 2.1.1

Immunocytochemistry

This was done as described in section 2.1.1.

The following incubation protocols were adopted: anti-c-Fos (1:100, 72 hrs at room temperature), anti-c-Jun (1:100, 48 hrs at 4 ^{O}C), anti-ATF-2 (1:150, 16 hrs at 4 ^{O}C), anti-Egr-1 (1:100, 48 hrs at room temperature), and anti-Egr-2 (1:50, 24 hrs at 4 ^{O}C). All the primary antibodies against immediate early gene proteins were from Santa Cruz Biotechnology, CA, USA. Sections were also stained with rabbit anti-TH (1:150, 24 hr at room temperature, Chemicon International, CA, USA).

Image analysis

This was done as described in section 2.1.1.

Statistical analysis

The data were analyzed by two-factor ANOVA for between-subjects effects of treatment duration and dose followed by post-hoc analysis with Tukey's Honestly Significantly Different (HSD) test. An alpha level of 0.05 was applied.

2.2 *CHAKRAGATI* MOUSE EXPERIMENTS

Subjects:

Experimental subjects were the genetically modified *chakragati* mice and heterozygous and wild type littermates. They were housed two to a cage during the duration of the stay in the animal holding unit. One week prior to the start of the experiment they were placed on a 23-hour water restriction schedule that continued throughout the experiment. During the days of the experimental procedure on which water was available in the test apparatus, this availability was in addition to the daily ration of the 1-hour given in the home cages. The animals were tested between 0800 and 1700hours. All experiments were approved by the institutional animal ethics review board of the National University of Singapore and were conducted in accordance with the International Guiding Principles for Animal Research (Howard-Jones, 1985)

2.2.1 LI

Apparatus

The apparatus consisted of a TSE operant behavior box (Model 259900-SK-MAU-ST/2). The box was operated in sound-attenuating housings equipped with a ventilation fan (model 259900-Hou-SK-M). The box had dimensions of 159 x 165 x 175 mm (LxWxH). The floor comprised of a "grid" through which electric shock could be applied to the test animal. The walls of the box could be fitted with various types of stimulus lights or sound stimulators. One of the walls housed the receptacle for the liquid dispenser. The dispenser itself was a drop-type dispenser featuring a software controlled magnetic valve that was opened for a user–defined length, allowing the experimenter to control the fluid volume

dispensed. Numbers of licks were monitored by infra-red sensors fitted at the opening of the receptacle. The pre-exposed, to be conditioned stimulus was a 2 watt white house light. Shock was delivered through the cage floor. It was supplied from the control unit, which was equipped with a microprocessor-controlled shocker scrambler module (model 259900-SHOCK). The scrambler was set to 0.1 mA. Equipment programming and data recording was computer controlled.

Procedure:

Pretreatment handling and the stages of the LI procedure are described below. The stages of preexposure, conditioning, rebaseline, and test were administered 24 hr apart.

Handling: Prior to the beginning of the experiment, the animals were handled for about 5 minutes everyday for 5 days and then put back into the cage.

Baseline: With the water flow on, for five days the mice were placed into the experimental chamber and allowed to drink water for 20 minutes.

Preexposure: The flow of water was stopped and then the mice were placed in the chamber. The preexposed (PE) group received forty 10 second house light exposures with a variable interstimulus interval (ISI) with a mean of 35 seconds. The non preexposed (NPE) animals were confined to the chamber for an identical period of time , but they did not receive the light stimuli.

Conditioning: With the water flow stopped, each animal received two light-shock pairings 5 and 10 minutes after the start of the session. The light parameters were identical to those used in preexposure. The 0.1 mA shock immediately followed the light

termination. After the second pairing, the animal was left in the experimental chamber for an additional 5 minutes.

Rebaseline: Each animal was given a drinking session similar to the baseline sessions. Latency to first lick and the total number of licks were recorded for each mouse.

Test: Each animal was placed in the chamber and allowed to drink water from the receptacle. When the animal completed 75 licks, the house light was presented for 5 minutes. The following time points were observed: time to first lick, time to complete 1-50 licks, time to complete 50-75 licks(pre light), latency to first lick after light presentation and the time to complete 75-100 licks (light on). Animals that failed to complete 25 licks within the 5 minute duration when the light was on were given a score of 300.

The amount of suppression of licking was measured using a suppression ratio, A/(A+B), where A was the period prior to the presentation of the houselight (licks 51-75) and B was the period of the house light presentation (licks 76-100). A suppression ration of 0.01 indicates complete suppression (no LI) and a suppression ratio of 0.50 indicates no change in response rate from the period prior to the presentation of the stimulus to the period of stimulus presentation (LI).

Statistical Analysis

Statistical analysis was done using a two-factor ANOVA (Exposure and genotype were the two fixed factors). Post hoc tests were done using the Tukey's test. P levels were fixed at 0.05.

2.2.2 PPI

Apparatus

The startle reactivity was measured using a startle chamber (SR-LAB, San Diego Instruments, San Diego, CA). The chamber consisted of a clear plexi glass cylinder resting on a platform inside a ventilated box. A high frequency loudspeaker inside the chamber produced both a continuous background noise of 65 db as well as the various acoustic stimuli. Vibrations of the plexi glass cylinder caused by the whole body startle response of the animal, were transduced into analog signals by a piezoelectric unit attached to the platform. These signals were then digitized and stored in a computer. The startle response was ascertained with the computer software.

The following experiments were conducted in the *ckr* mice to test the PPI.

a) Validation Experiment: A "schizophrenia model" validation experiment was conducted where three different types of genotypic mice were tested (wild type, heterozygous and homozygous *ckr*).

Statistical analysis:

Data were analyzed by a Two-way ANOVA with genotype as between subjects factor and the trial type (prepulse intensity) as a repeated measure. Post hoc tests were carried out using Tukey's test. P level was set at 0.05. Startle magnitude was calculated as the average response to all of the PULSE-ALONE trials. Startle magnitude significance was analyzed with a one way ANOVA.

b) Hearing Defect Experiment: This was followed by an experiment so as to ascertain whether the homogenous *ckr* mice had any hearing defect. In this the time duration between the prepulse and pulse tones was varied. Three different time durations between prepulse and pulse tones was used (25ms, 100 ms and 175 ms)]

Statistical analysis:

This was done for double repeated measures (time gaps and prepulse intensity) for the homozygous mice.

c) Drug Experiments: Finally the effect of antipsychotic drugs and adrenergic drugs were tested on the *ckr* mice.

- Phase I: PPI was tested after three different doses of Clozapine (1/4/10 mg/kg s.c), Risperidone (0.1/0.5/1.0 mg/kg s.c) and Haloperidol (0.1/0.5/1.0 mg/kg s.c) were administered to the animals.
- Phase II: PPI was tested for effect of alpha-1 adrenergic agonist drug cirazoline (0.75 mg/kg s.c) and alpha-1 adrenergic antagonist drug Prazosin (1.0 mg/kg i.p) alone. Then PPI was tested for combined effect of clozapine (1/4/10 mg/kg s.c) dosages and alpha-1 antagonist drug Prazosin (1.0 mg/kg i.p).

The drugs haloperidol (Sigma), clozapine (Tocris) and risperidone (Sigma) were dissolved in distilled water acidified to pH 4.5 to 5 with acetic acid. Cirazoline (Tocris) was dissolved in 0.9 % saline. Prazosin (Tocris) was dissolved in a vehicle solution of isotonic saline-propylene glycol-ethanol (5:4:1).

Procedure

The following broad based protocol was followed for the PPI experiments:

Acclimatization: The animals were left in the plexi glass chamber for 5 minutes to get acclimatize. During this period a background noise of 65 db was present.

Trials: Five different types of trials were conducted:

- **Pulse alone**: A stimuli of 120 db is provided for 40 ms.
- **Pulse + Prepulse**: A 68 db (+3) stimuli for 20 ms precedes the 120 db stimuli by 100 ms.
- **Pulse + Prepulse**: A 71 db (+6) stimuli for 20 ms precedes the 120 db stimuli by 100 ms.
- **Pulse + Prepulse**: A 77 db (+12) stimuli for 20 ms precedes the 120 db stimuli by 100 ms.
- **No pulse**: Just the background noise is present.

[The + sign denotes the difference between the prepulse and the background noise intensity.]

In one session a total of 52 trials are conducted in pseudorandom order: 20 Pulse Alone trials, and 8 each of the other four trials. These are preceded by 4 Pulse Alone trials, which are discarded. An average of 15 s was kept as Inter-Trial Interval (9-21 s range). For the hearing defect experiment, the time duration between prepulse and pulse stimuli were altered as mentioned above.

The data was used to find out two parameters:

- **Pre Pulse Inhibition**: This is given by the formula

  (Pulse – Prepulse)

  ---------- x 100

  Pulse

- **Startle Amplitude**: Measure of the average startle for Pulse Alone trials.

Statistical analysis

Because the prepulse intensity factor did not interact significantly with any other factor in the experiment, this factor was collapsed by averaging the PPI values for the different prepulse intensities, there by creating a global PPI score for each mice. All PPI and startle magnitude data were analysed with two-factor ANOVA where the genotype and the drug treatments were the fixed factors. Post hoc analysis was conducted using Tukey's test. The α level was set to 0.05.

2.3 RAT EXPERIMENTS

The same sets of animals were used in both the rat behavior experiments of PPI and Water Maze.

2.3.1 Subjects

Experimental subjects were male SD rats weighing 200-250 gm at the time of testing. They were housed four to a cage during the duration of the stay in the animal holding unit.

They were maintained in a humidity and temperature controlled room with a constant 12 hour light / dark cycle (lights on at 7.00 am). Food and water were freely available throughout the experiments except during the behavior testing. The animals were allowed to habituate to the animal maintenance facilities for a period of at least one week before the initiation of the experiments. During this time the experimenter handled the animals gently every day so as to minimize the stress during the testing. The animals were brought to the experiment room in plastic cages and were allowed to have a habituation period of 60 minutes in the room for the first day and 30 minutes on the following days. The animals were tested between 0800 and 1600 hours. All experiments were approved by the institutional animal ethics review board of the National University of Singapore and were conducted in accordance with the International Guiding Principles for Animal Research (Howard-Jones, 1985).

2.3.2 Drug Treatment

Both PPI and WM were conducted in two phases. In phase I, I tried to compare the differences between four groups (n=8) which were given the following drug treatments:

i) Haloperidol (0.5 mg/kg/day) for 4 weeks and MK-801 (0.1 mg/kg) on the day of the testing.

ii) Olanzapine (10 mg/kg/day) for 4 weeks and MK-801 (0.1 mg/kg) on the day of the experiment.

iii) MK-801 (0.1 mg/kg) on the day of the testing.

iv) Saline injection for 4 weeks as well as on the day of the testing.

In phase II we tested four groups of animals (n=8), these were given the following drug treatments:

i) Olanzapine (10 mg/kg/day) and alpha-1 agonist drug Cirazoline (0.75 mg/kg/day s.c) for 4 weeks and MK-801 (0.1 mg/kg s.c) on the day of the testing.

ii) Olanzapine (10 mg/kg/day s.c) and alpha-1 antagonist drug Prazosin (1.0 mg/kg/day i.p) for 4 weeks and MK-801 (0.1 mg/kg) on the day of the testing.

iii) Olanzapine (10 mg/kg/day s.c) and alpha-2 agonist drug Clonidine (0.2 mg/kg/day i.p) for 4 weeks and MK-801 (0.1 mg/kg) on the day of the testing.

iv) Olanzapine (10 mg/kg/day s.c) and alpha-2 antagonist drug Idazoxan (1.5 mg/kg/day s.c) for 4 weeks and Mk-801 (0.1 mg/kg) on the day of the testing.

Haloperidol (Sigma) was dissolved in distilled water acidified to pH 4.5 to 5 with acetic acid. Olanzapine (Eli Lilly and Company, Indianapolis, IN, USA) was dissolved in distilled water acidified to pH 5.5 by application of 1 M HCl and adjusted back to pH 6.0 with 1 M NaOH. Saline (0.9% NaCl in distilled water) was acidified to pH 6.0 by application of 1 M HCl. MK-801 ((+)-10.11-dihydro-5-methyl-5H-dibenzo [a,d]-cyclohepten-5, 10-imine hydrogen maleate) (Tocris) , Clonidine (Tocris), Idazoxan (Sigma) and Cirazoline (Tocris) were dissolved in 0.9 % saline. Prazosin (Tocris) was dissolved in a vehicle solution of isotonic saline-propylene glycol-ethanol (5:4:1). Chronic daily dosages were administered between 16:00 h and 17:00 h.

2.3.3 PPI Study

Apparatus

The apparatus used was same as described before in section 2.2.2.

Procedure

The procedure followed was same as described before in the section 2.2.2.

Statistical analysis

Because the prepulse intensity factor did not interact significantly with any other factor in the experiment, this factor was collapsed by averaging the PPI values for the different prepulse intensities, there by creating a global PPI score for each rat. All PPI and startle magnitude data were analyzed with two-factor ANOVA where the treatment duration (1 week, 2 week or 4 week) and the drug treatments were the fixed factors. Post hoc analysis was conducted using Tukey's test. The α level was set to 0.05.

2.3.4 Water Maze Study

Apparatus

The rats were tested in a black circular pool (190 cm diameter, 60 cm height). The pool was filled with water, which was made opaque by adding non irritant ink to the water. The temperature of the water was maintained at 24 ± 2 C. An escape platform (18 x 18 cm) stood 2 cm below the water surface and provided the only escape form the water. The pool was located in the center of the room, which contained several extramazal cues

for the animal. These cues were kept constant throughout the experiment. A digital system consisting of CCTV camera was attached to the roof of the room above the center of the pool. The camera was connected to a computer system carrying the software system EthoVision version 2.3.19 (Noldus Information Technology BV, Wageningen, The Netherlands). The parameters monitored were latency to the escape platform, swim distance and swim speed.

Procedure

Once a day the rats received the respective antipsychotic (Haloperidol or Olanzapine) and adrenergic drug (Prazosin, Cirazoline, Clonidine or Idazoxan) injections. The drug schedule followedwas asdescribed in section 2.3.2. Thirty minutes before the experiment, the animals received either MK-801 or saline injections. The pool was divided into four equal sized quadrants. These were designated as north, east, south and west. On day 1, the first trial started from starting point "East". The starting points varied over the next four days of training, and they were rotated clockwise, one quarter of a turn per trial (Ahlander et. al., 1999). The position of the platform was fixed and it remained in the center of the southwest quadrant throughout the training.

For each trial the animal was gently lowered in the water with its face towards the wall of the pool. The animal was allowed to swim for 60 seconds. If the animal failed to locate the platform within this time period, it was guided by hand towards the platform. They were then allowed to rest on the platform for about 30 s. after each trial (Inter trial interval). After the last trial they were put in a drying cage and allowed to dry for about 5

minutes. They were then returned to their experimental cages. At the end of the session the animals were brought back to the animal house and returned to their home cages.

Statistical analysis

A nested repeated measures analysis of variance was used to examine the difference between treatments within day and differences between days within treatment for three outcomes measures: latency, distance and swim speed. For each ANOVA model, fixed effects included day (1, 2, 3 and 4) and treatment (control, MK-801, MK-801 + Haloperidol, MK-801 + Olanzapine). The two-factor interaction between treatment and day was also included in the model. Tukey's post-hoc tests were further conducted with P level at 0.05.

RESULTS

3.1 IMMUNOHISTOCHEMISTRY EXPERIMENTS

3.1.1 Comparison between typical and atypical antipsychotics

c-Fos immunoreactivity

The antipsychotic drug treatment for 4 weeks significantly influenced the expression of c-Fos in the mPFC (one-way ANOVA, $F_{3,28} = 37.32$, $p < 0.001$; **Fig. 2a**) but not in the LC (one-way ANOVA, $F_{3,28} = 0.667$, n.s.; **Fig. 2b).** Haloperidol significantly reduced the number of c-Fos immunopositive nuclei (post-hoc Dunnett's test, $p < 0.01$), while clozapine increased the number of c-Fos immunopositive nuclei (post-hoc Dunnett's test, $p < 0.001$). Risperidone did not significantly change number of c-Fos positive nuclei.

Egr-1 immunoreactivity

The 4-weeks antipsychotic drug treatment significantly influenced the expression of Egr-1 in both the mPFC (one-way ANOVA, $F_{3,28} = 4.472$, $p < 0.05$; **Fig. 6a**) and the LC (one-way ANOVA, $F_{3,28} = 5.213$, $p < 0.01$; **Fig. 6b**). Post-hoc Dunnett's tests against the acidified saline-treated control group revealed that in the mPFC the effect was largely attributable to the significant increase in Egr-1 expression on treatment with haloperidol ($p < 0.01$; **Fig. 6a**). Neither risperidone nor clozapine had any significant effect on Egr-1 expression in the mPFC. In contrast in the LC, all three antipsychotics reduced Egr-1 expression (haloperidol, $p < 0.001$; risperidone, $p < 0.05$; clozapine, $p < 0.05$; **Fig. 6b**).

Egr-2 immunoreactivity

In the mPFC, there was a general trend towards upregulation of Egr-2 expression following 4-weeks treatment with all three antipsychotic drugs (**Fig. 7a**), but this did not reach significance (one-way ANOVA, $F_{3,28} = 5.213$, n.s.). In the LC, drug treatment for 4 weeks resulted in a significant downregulation of Egr-2 expression (one-way ANOVA, $F_{3,28} = 11.337$, $p < 0.001$; **Fig. 7b**). Post-hoc Dunnett's test comparisons with the acidified saline-treated control group revealed that all three drugs significantly reduced the number of Egr-2 immunopositive nuclei (haloperidol, $p < 0.001$; risperidone, $p < 0.005$; clozapine, $p < 0.01$; **Fig. 7b**).

TH immunoreactivity

In both the mPFC and LC, 4-weeks treatment with the antipsychotic drugs significantly influenced TH immunoreactivity in both the mPFC (one-way ANOVA, $F_{3,28} = 91.944$, $p < 0.001$; **Fig. 10a**) and the LC (one-way ANOVA, $F_{3,28} = 420.873$, $p < 0.001$, **Fig 10b**). In the mPFC, post-hoc Dunnett's test comparisons against the acidified saline-treated control group showed that risperidone ($p < 0.001$) and clozapine ($p < 0.001$) both significantly increased TH immunoreactivity. In the LC, all three antipsychotic drugs increased TH immunoreactivity compared to the acidified saline-treated control group (post-hoc Dunnett's tests, $p < 0.001$ for all three drugs). However, post-hoc Tukey HSD comparisons to the haloperidol-treated group revealed that risperidone and clozapine ($p < 0.001$, in both cases) resulted in greater increases in counts of TH immunopositive profiles. Clozapine in turn produced greater increases in TH expression than risperidone (Tukey HSD test, $p < 0.001$).

a) **b)**

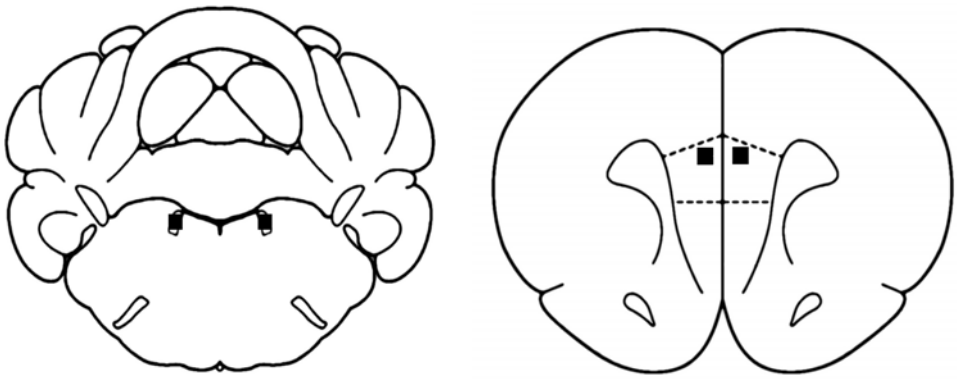
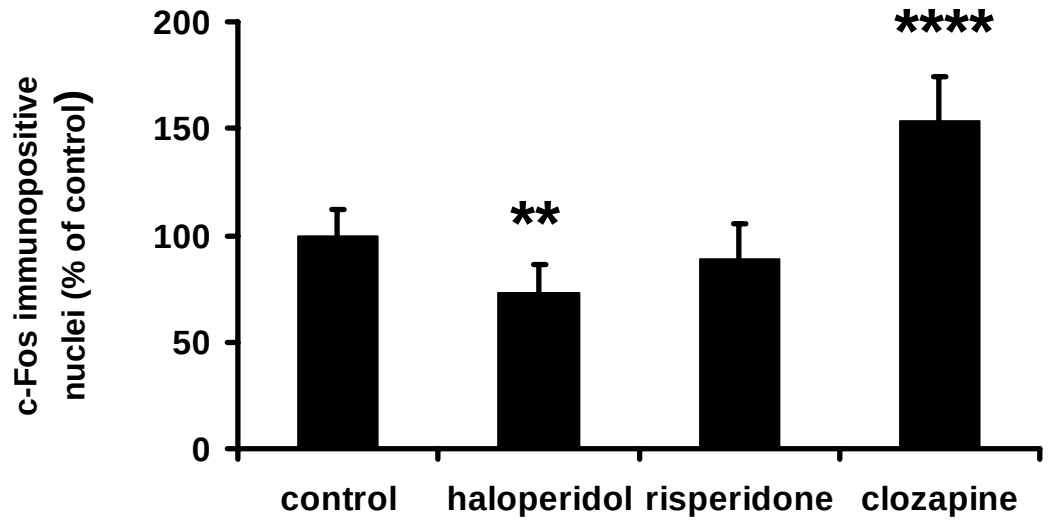


Fig. 1: Drawings of representative sections showing (boxes) the regions of the a) prelimbic (PrL) area of the medial prefrontal cortex and b) Locus Coeruleus (LC) sampled.The drawings are adapted from Paxinos and Watson (1997).

(a) Medial Prefrontal cortex



(b) Locus coeruleus

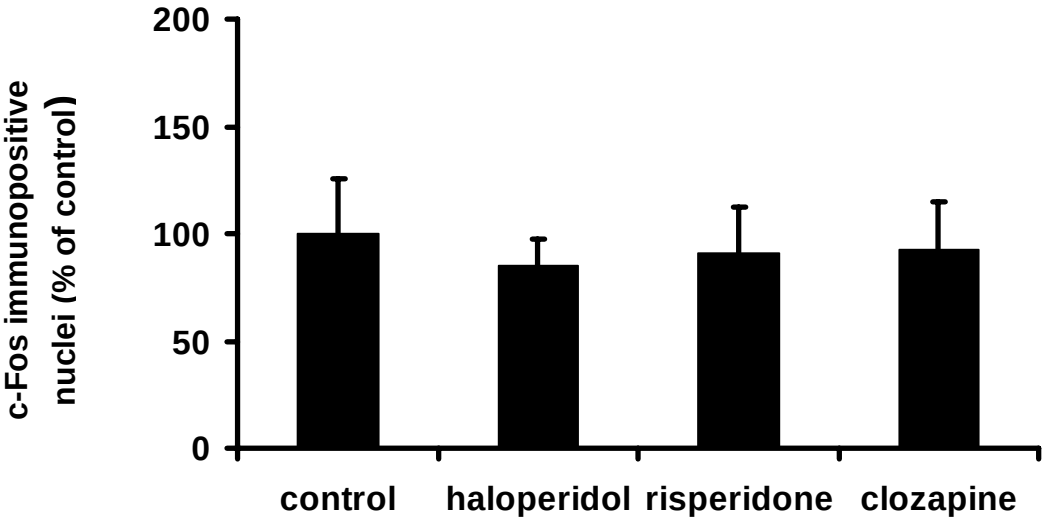


Fig. 2. Effects of 4-week antipsychotic drug treatment on c-Fos expression in (a) the medial prefrontal cortex (mPFC) and (b) the locus coeruleus (LC). The data are the number of c-Fos immunopositive nuclei expressed as a percentage of the number in the acidified saline-treated control group (mean ± std). There was a significant treatment effect (one-way ANOVA, $p < 0.001$) in the mPFC but not in the LC. Post-hoc Dunnett's tests against the acidified saline-treated control group revealed significant effects of haloperidol in the mPFC (** $p < 0.01$) and clozapine (**** $p < 0.001$) in the mPFC.

a) **b)**

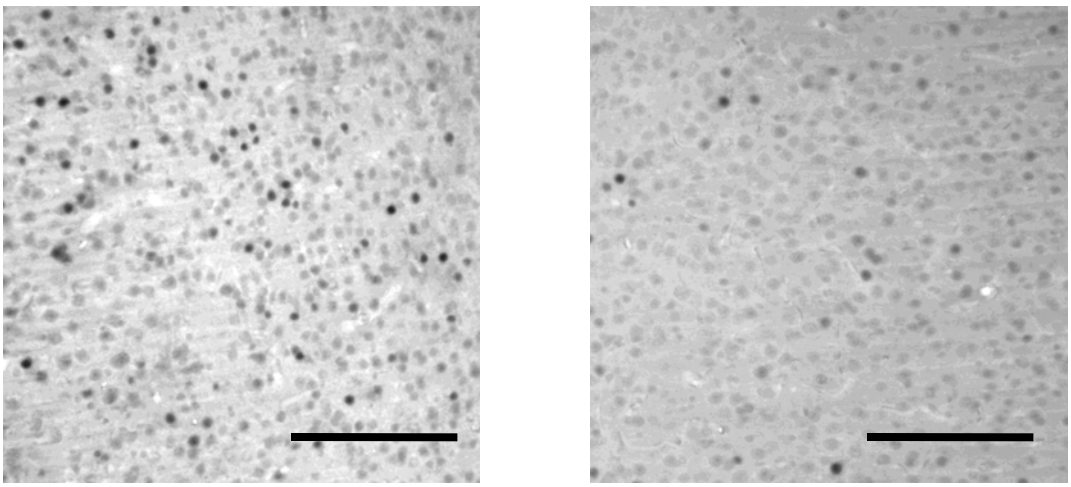


Fig. 3: Showing c-Fos Expression with a) Clozapine and b) Haloperidol.

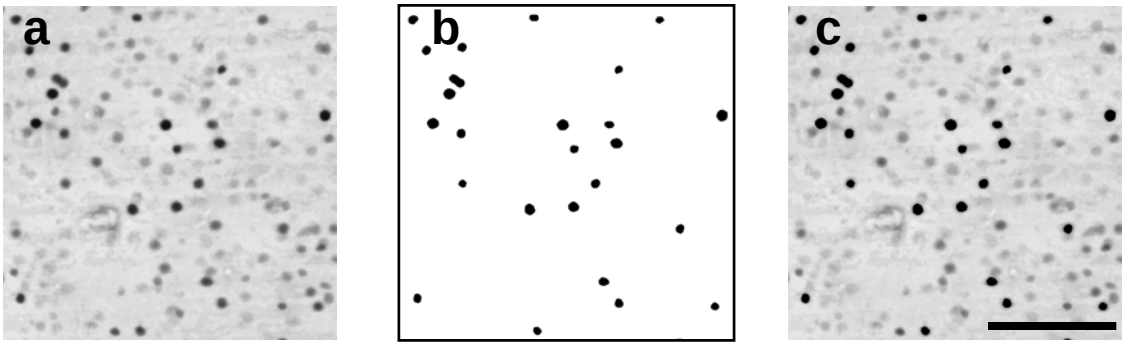


Fig. 4: Counting of c-Fos immunoreactive nuclei. **(a)** A representative photomicrograph stained with anti-c-Fos antibody. **(b)** The same image as in (a) after selection of activated, intensely staining immunopositive nuclei by binary thresholding and application of object size and roundness filters. **(c)** The same image as in (b) superimposed on the original photomicrograph shown in (a) to illustrate the accuracy of the selection of intensely stained c-Fos immunopositive nuclei. The scale bar represents 75 μm.

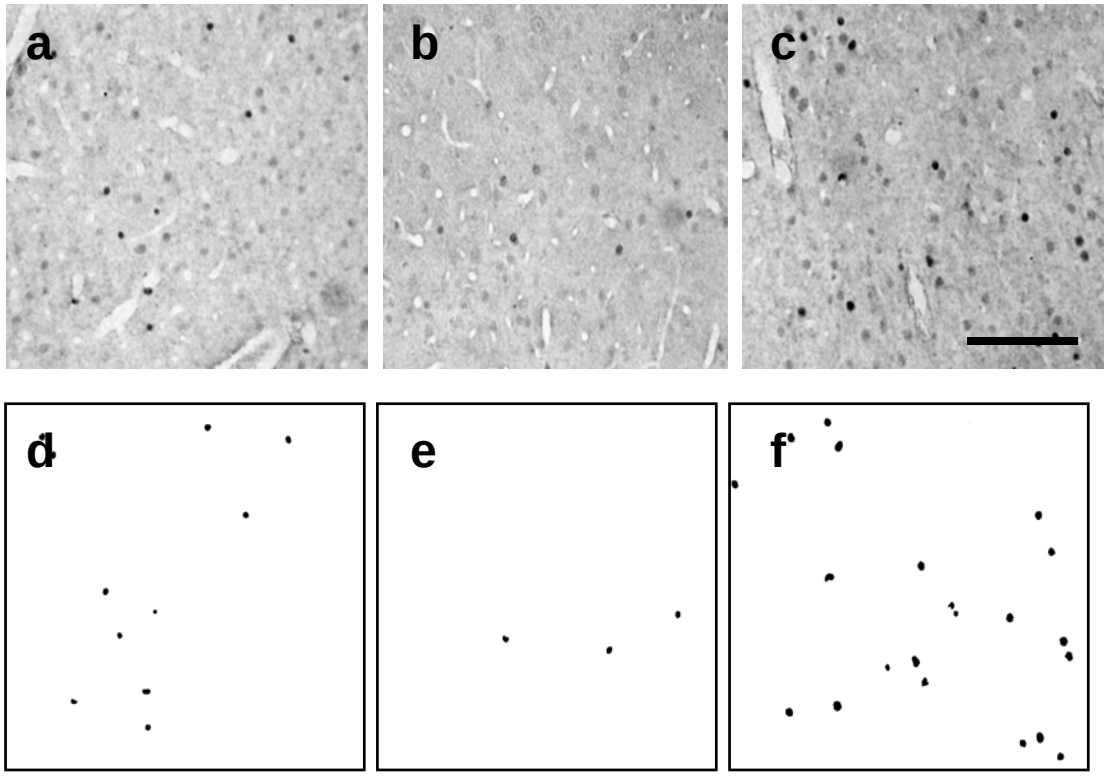
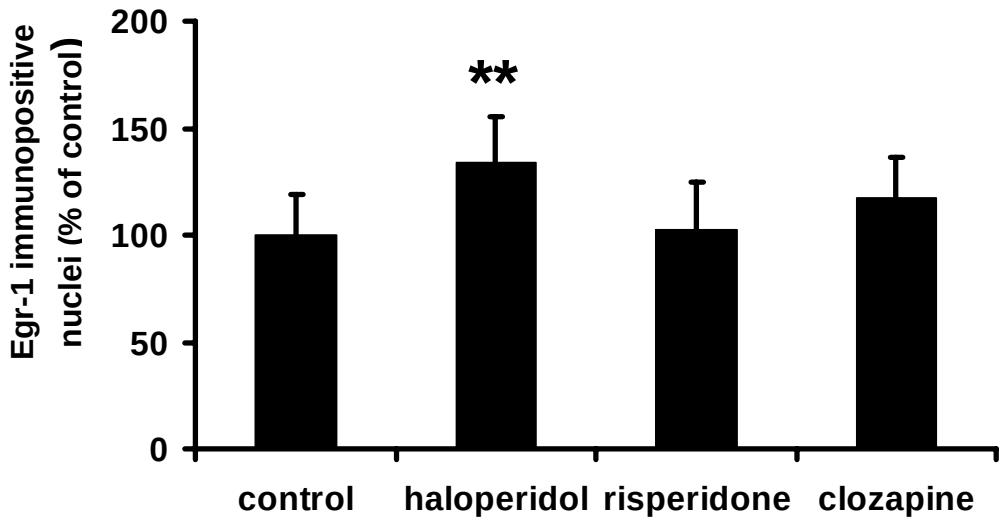


Fig. 5: Representative examples of photomicrographs of immunostaining with anti-c-Fos antibody in the mPFC following chronic treatment. Sections from matched regions of the mPFC of animals treated for 4 weeks with (a) acidified saline, (b) haloperidol and (c) clozapine. To select immunopositive nuclei for counting the images were processed by binary thresholding, binary erosion and dilation filtering, and application of object size and roundness filters. The same images as in (a)–(c) are shown after image process in (d)–(f), respectively. The scale bar represents 200 mm.

(a) Medial Prefrontal cortex



(b) Locus coeruleus

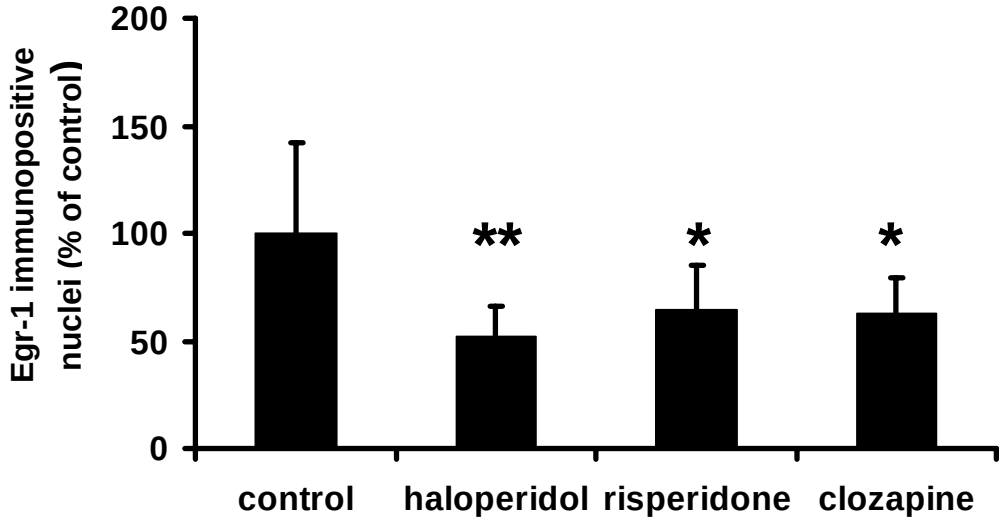
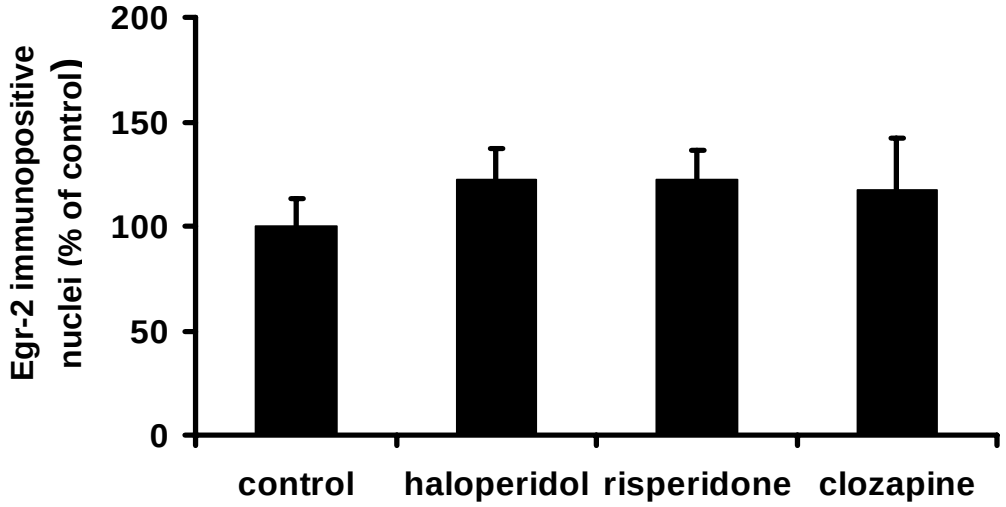


Fig. 6: Effects of 4-week antipsychotic drug treatment on Egr-1 expression in (a) the medial prefrontal cortex (mPFC) and (b) the locus coeruleus (LC). The data are the number of Egr-1 immunopositive nuclei expressed as a percentage of the number in the acidified saline-treated control group (mean ± std). There were significant treatment effects in both the mPFC (one-way ANOVA, $p < 0.05$) and LC (one-way ANOVA, $p < 0.01$). * $p < 0.05$, and ** $p < 0.01$ on post-hoc Dunnett's test comparisons with the acidified saline-treated control group.

(a) Medial Prefrontal cortex



(b) Locus coeruleus

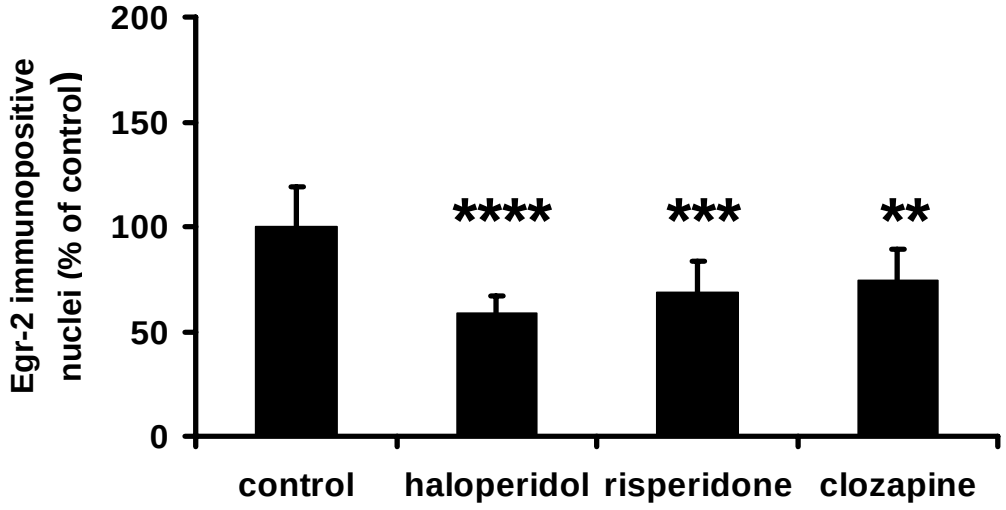
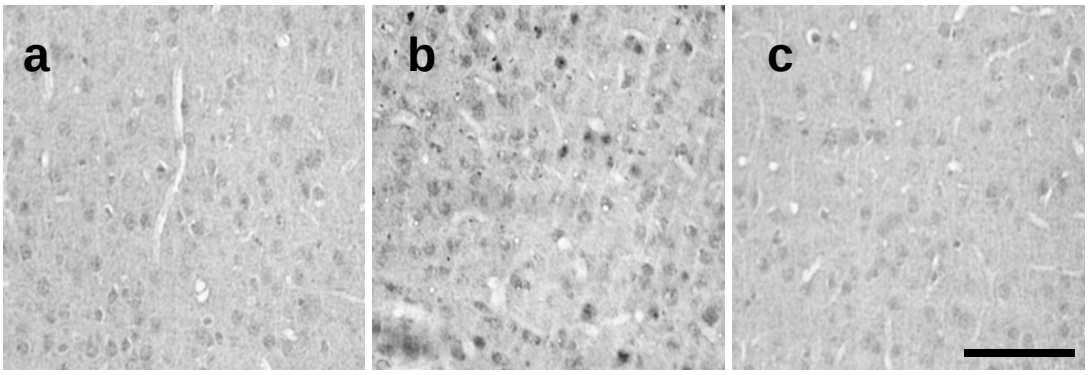
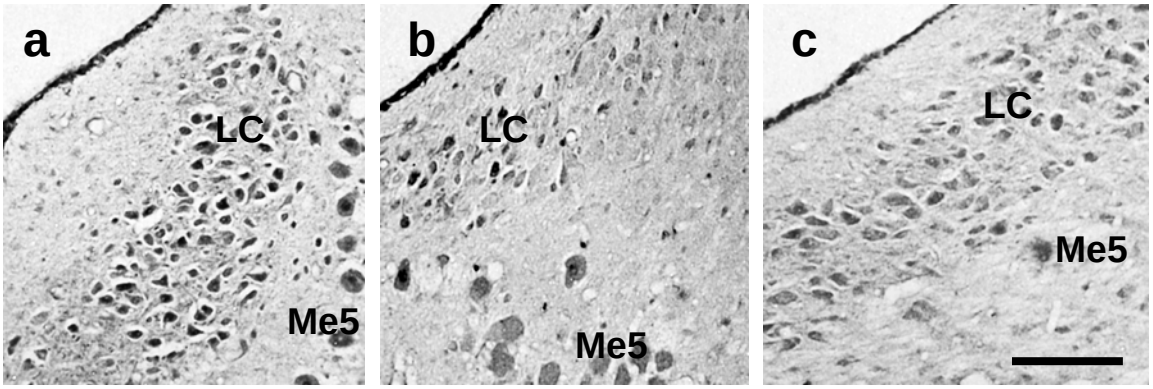


Fig. 7: Effects of 4-week antipsychotic drug treatment on Egr-2 expression in (a) the medial prefrontal cortex (mPFC) and (b) the locus coeruleus (LC). The data are the number of Egr-2 immunopositive nuclei expressed as a percentage of the number in the acidified saline-treated control group (mean ± std). There was a significant treatment effect in LC (one-way ANOVA, $p < 0.001$) but not in the mPFC (one-way ANOVA, n.s.). ** $p < 0.01$, *** $p < 0.005$, and **** $p < 0.001$ on post-hoc Dunnett's test comparisons with the acidified saline-treated control group.



Acidified Saline **Haloperidol** **Clozapine**

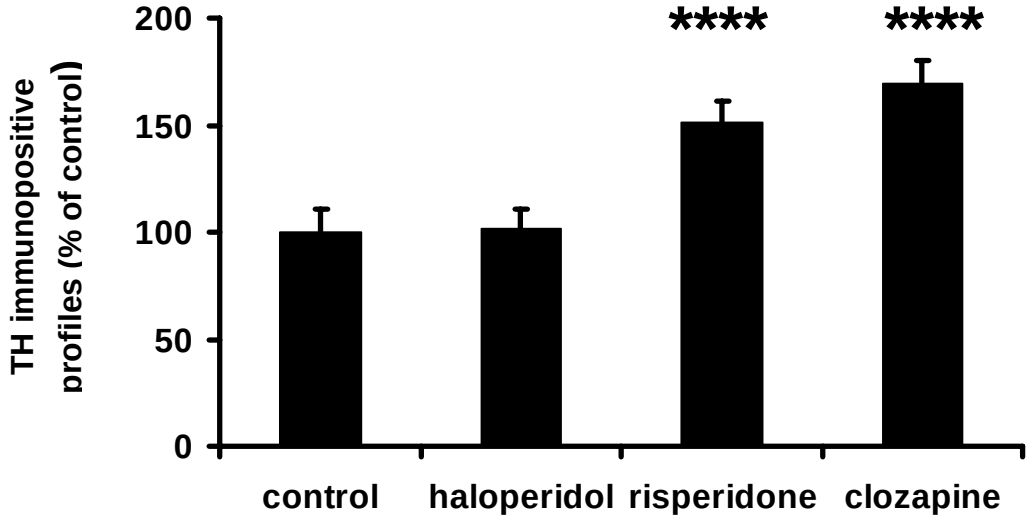
Fig. 8: Representative examples of photomicrographs of immunostaining with anti-Egr-1 antibody in the mPFC. Sections from matched regions of the mPFC of animals treated for 4 weeks with (a) acidified saline, (b) haloperidol and (c) clozapine. The scale bar represents 200 mm.



Acidified Saline **Halopreidol** **Clozapine**

Fig. 9: Representative examples of photomicrographs of immunostaining with anti-Egr-2 antibody in the LC. Sections containing the LC of animals treated for 4 weeks with (a) acidified saline, (b) haloperidol and (c) clozapine. The IVth ventricle in the upper left-hand side of the photomicrographs and the large cells of the mesencephalic nucleus of the Vth nerve (Me5) lateral and ventral to the LC serve as landmarks for locating the LC. The scale bar represents 200 mm.

(a) Medial Prefrontal cortex



(b) Locus coeruleus

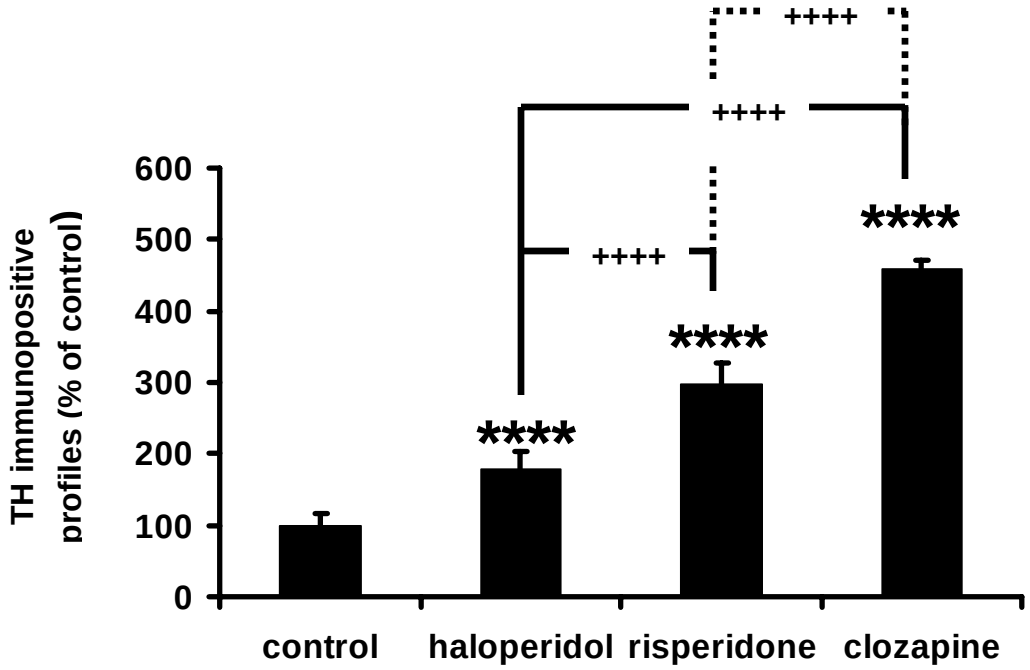


Fig. 10: Effects of 4-week antipsychotic drug treatment on tyrosine hydroxylase (TH) expression in (a) the medial prefrontal cortex (mPFC) and (b) the locus coeruleus (LC). The data are the number of TH immunopositive profiles expressed as a percentage of the number in the acidified saline-treated control group (mean ± std). There were significant treatment effects both the mPFC (one-way ANOVA, $p < 0.001$) and LC (one-way ANOVA, $p < 0.001$). **** $p < 0.001$ on post-hoc Dunnett's test comparisons with the acidified saline-treated control group. $^{++++}$ $p < 0.001$ on Tukey's HSD test between drug treatment groups.

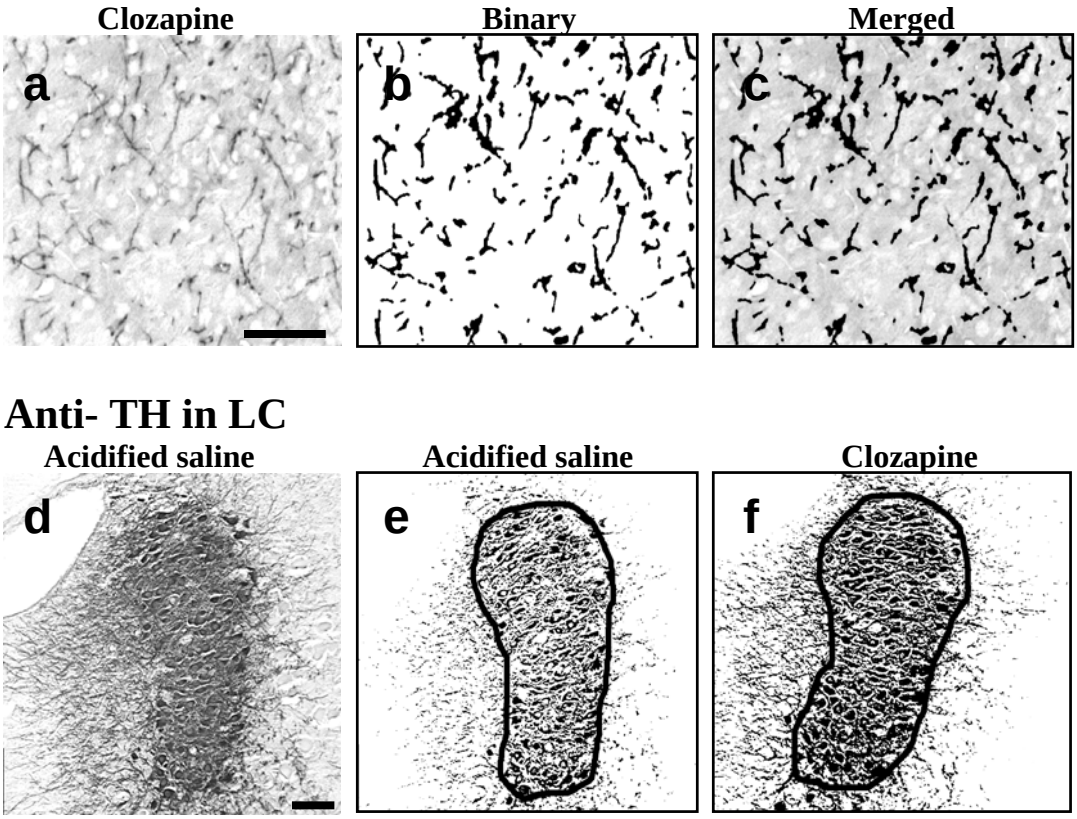


Fig. 11: Illustration of the immunostainning for TH. (a) A representative photomicrograph of immunostaining with anti-TH antibody in the mPFC of an animal treated with clozapine. (b) The same image as in (a) after selection of immunopositive fibre profiles by binary thresholding, binary erosion and dilation filtering, and application of object size filters. (c) The same image as in (b) superimposed on the original photomicrograph shown in (a) to illustrate the accuracy of the selection of TH-immunopositive fibre profiles. (d) A representative photomicrograph of immunostaining with anti-TH antibody in the LC of an animal treated with acidified saline. (e) The same image as in (d) after selection of immunopositive profiles of neurites and cell bodies by binary thresholding, binary erosion and dilation filtering, and application of object size and roundness filters. The bold black line encircling the LC illustrates the region defined as the LC for the purpose of counting the TH immunopositive profiles and measuring the area of the LC. (f) A representative example of an image from an animal treated with clozapine subjected to the same image processing. The scale bars represent 100 μm.

3.1.2 Effect of different dosages and treatment durations of olanzapine on IEG and TH expression

c-Fos immunoreactivity

Two-way ANOVA of c-Fos immunoreactivity revealed significant main effects of dose and duration of olanzapine treatment, as well as an interaction of these two factors, in both the LC (dose, $F_{4, 60} = 68.0$, $p < 0.0001$; duration, $F_{3, 60} = 465$, $p < 0.0001$; dose x duration interaction, $F_{12, 60} = 43.2$, $p < 0.0001$) and mPFC (dose, $F_{4, 60} = 19.45$, $p < 0.0001$; duration, $F_{3, 60} = 32.5$, $p < 0.0001$; dose x duration interaction, $F_{12, 60} = 9.54$, $p < 0.0001$). Post-hoc Tukey HSD tests confirmed that in both LC and mPFC there was a dose-dependent increase in c-Fos immunoreactivity for 4-hour, 1-week, and 2-week durations of treatment (**Figure 13**). In the LC, the increase in c-Fos immunoreactivity induced by the doses 2/4/8 mg/kg/day did not reach significance until 2-weeks treatment. However, on 4-weeks treatment a down regulation of c-Fos immunoreactivity was observed in both LC and mPFC (**Figure 13**).

c-Jun immunoreactivity

Two-way ANOVA of c-Jun immunoreactivity revealed significant main effects of dose and duration of olanzapine treatment, as well as an interaction of these two factors, in the both the LC (dose, $F_{4, 60} = 122$, $p < 0.0001$; duration, $F_{3, 60} = 15.2$, $p < 0.0001$; dose x duration interaction, $F_{12, 60} = 7.89$, $p < 0.0001$) and mPFC (dose, $F_{4, 60} = 649$, $p < 0.0001$; duration, $F_{3, 60} = 487$, $p < 0.0001$; dose x duration interaction, $F_{12, 60} = 112$, $p < 0.0001$). Post-hoc Tukey HSD tests confirmed that in both LC and mPFC there was a dose-

dependent increase in c-Jun immunoreactivity for 4 hrs, 1 week, and 2 weeks durations of treatment (**Figure 14**). For the lowest dose, 2 mg/kg/day, consistent increases in the number of c-Jun immunoreactive cells were only detected after 2-weeks treatment. The pattern of dose-dependent increases in c-Jun immunoreactivity continued with 4-weeks treatment in the LC, but in the mPFC on 4-weeks treatment a down regulation of c-Jun immunoreactivity was observed which significantly reduced immunoreactivity below the control level for the highest doses, 15 mg/kg/day (**Figure 14**).

ATF-2 immunoreactivity

Two-way ANOVA of ATF-2 immunoreactivity revealed significant main effects of dose and duration of olanzapine treatment, as well as an interaction of these two factors, in the both the LC (dose, $F_{4,60} = 1402$, $p < 0.0001$; duration, $F_{3,60} = 13.9$, $p < 0.0001$; dose x duration interaction, $F_{12,60} = 4.6$, $p < 0.0001$) and mPFC (dose, $F_{4,60} = 208$, $p < 0.0001$; duration, $F_{3,60} = 50.7$, $p < 0.0001$; dose x duration interaction, $F_{12,60} = 63.8$, $p < 0.0001$). Post-hoc Tukey HSD tests confirmed that in the LC all doses had increased ATF-2 immunoreactivity by 4 hrs and that the number of ATF-2 immunoreactive cells was sustained at all treatment durations (**Figure 15**). In the mPFC, post-hoc Tukey HSD tests revealed that the lowest dose, 2 mg/kg/day, decreased the number of cells expressing ATF-2 following 4 hrs and 1 week treatments but increased ATF-2 immunoreactivity at 2 weeks and 4 weeks treatments (**Figure 15**). Higher doses increased ATF-2 immunoreactivity even at the 4 hr time-point, but on treatment with the highest dose, 15 mg/kg/day, ATF-2 expression reduced to control levels again by 2 weeks treatment (**Figure 15**).

Egr-1 immunoreactivity

Two-way ANOVA of Egr-1 immunoreactivity revealed significant main effects of dose and duration of olanzapine treatment, as well as an interaction of these two factors, in the both the LC (dose, $F_{4,60} = 271$, $p < 0.0001$; duration, $F_{3,60} = 12.7$, $p < 0.0001$; dose x duration interaction, $F_{12,60} = 17.2$, $p < 0.0001$) and mPFC (dose, $F_{4,60} = 309$, $p < 0.0001$; duration, $F_{3,60} = 41.9$, $p < 0.0001$; dose x duration interaction, $F_{12,60} = 5.80$, $p < 0.0001$). Post-hoc Tukey HSD tests confirmed that in both LC and mPFC there was a down-regulation of Egr-1 immunoreactivity at all treatment durations (**Figure 16**). In both the LC and the mPFC, the down-regulation of Egr-1 immunoreactivity was inversely related to dose and greatest at lower doses. In the LC, the down-regulation was greatest on 1-week treatment with 2 mg/kg/day and decreased with longer treatment durations at this dose (**Figure 16**). In the mPFC, the down-regulation was also greatest on treatment with 2 mg/kg/day, but was similar with 4 hr, 1-week and 2-week treatments, and slightly greater with 4-weeks treatment.

Egr-2 immunoreactivity

Two-way ANOVA of Egr-2 immunoreactivity revealed significant main effects of dose and duration of olanzapine treatment, as well as an interaction of these two factors, in the both the LC (dose, $F_{4,60} = 526$, $p < 0.0001$; duration, $F_{3,60} = 11.0$, $p < 0.0001$; dose x duration interaction, $F_{12,60} = 12.6$, $p < 0.0001$) and mPFC (dose, $F_{4,60} = 916$, $p < 0.0001$; duration, $F_{3,60} = 58.3$, $p < 0.0001$; dose x duration interaction, $F_{12,60} = 32.1$, $p < 0.0001$). Post-hoc Tukey HSD tests confirmed that in both LC and mPFC there was a dose-dependent down-regulation of Egr-2 immunoreactivity at all treatment durations (**Figure 17**) . In the LC the down regulation was significant at all doses and treatment durations.

In the mPFC, the down-regulation was significant only at the higher doses (8 and 12 mg/kg/day) for all but the 4-week treatment duration (**Figure 17**).

TH immunoreactivity

Two-way ANOVA of TH immunoreactivity revealed significant main effects of dose and duration of olanzapine treatment, as well as an interaction of these two factors, in the both the LC (dose, $F_{4,60} = 447$, $p < 0.0001$; duration, $F_{3,60} = 13.0$, $p < 0.0001$; dose x duration interaction, $F_{12,60} = 4.6$, $p < 0.0001$) and mPFC (dose, $F_{4,60} = 61.2$, $p < 0.0001$; duration, $F_{3,60} = 61.4$, $p < 0.0001$; dose x duration interaction, $F_{12,60} = 9.4$, $p < 0.0001$). Post-hoc Tukey HSD tests confirmed that in the LC all doses and durations of treatment increased TH immunoreactivity (**Figure 18**). The increases in TH immunoreactivity were most marked for treatment with 4 mg/kg/day, while lower and higher doses produced less significant changes. While doses of 4 mg/kg/day and above produced similar increases in TH immunoreactivity at all treatment durations (**Figure 18**), post-hoc Tukey HSD tests suggest that the significance of the dose x duration interaction in the LC may be attributable a duration-dependent reduction in the increase TH immunoreactivity seen on treatment with 2 mg/kg/day olanzapine (320 ± 24.3 % increase at 4 hrs compared with 151 ± 7.0 % increase at 4 weeks, $P < 0.001$).

Post-hoc Tukey HSD tests showed that in mPFC that the increase TH immunoreactivity was both duration-dependent and dose-dependent (**Figure 18**). The greatest increases in TH immunoreactivity were seen on treatment with 8 mg/kg/day olanzapine. With doses of 8 and 15 mg/kg/day of olanzapine there were significant increases in TH immunoreactivity only after 2-weeks treatment. Treatment with lower doses (2 and 4

mg/kg/day) only produced significant increase in immunoreactivity after 4-weeks treatment (**Figure 18**).

The figures for this experiment are given below.

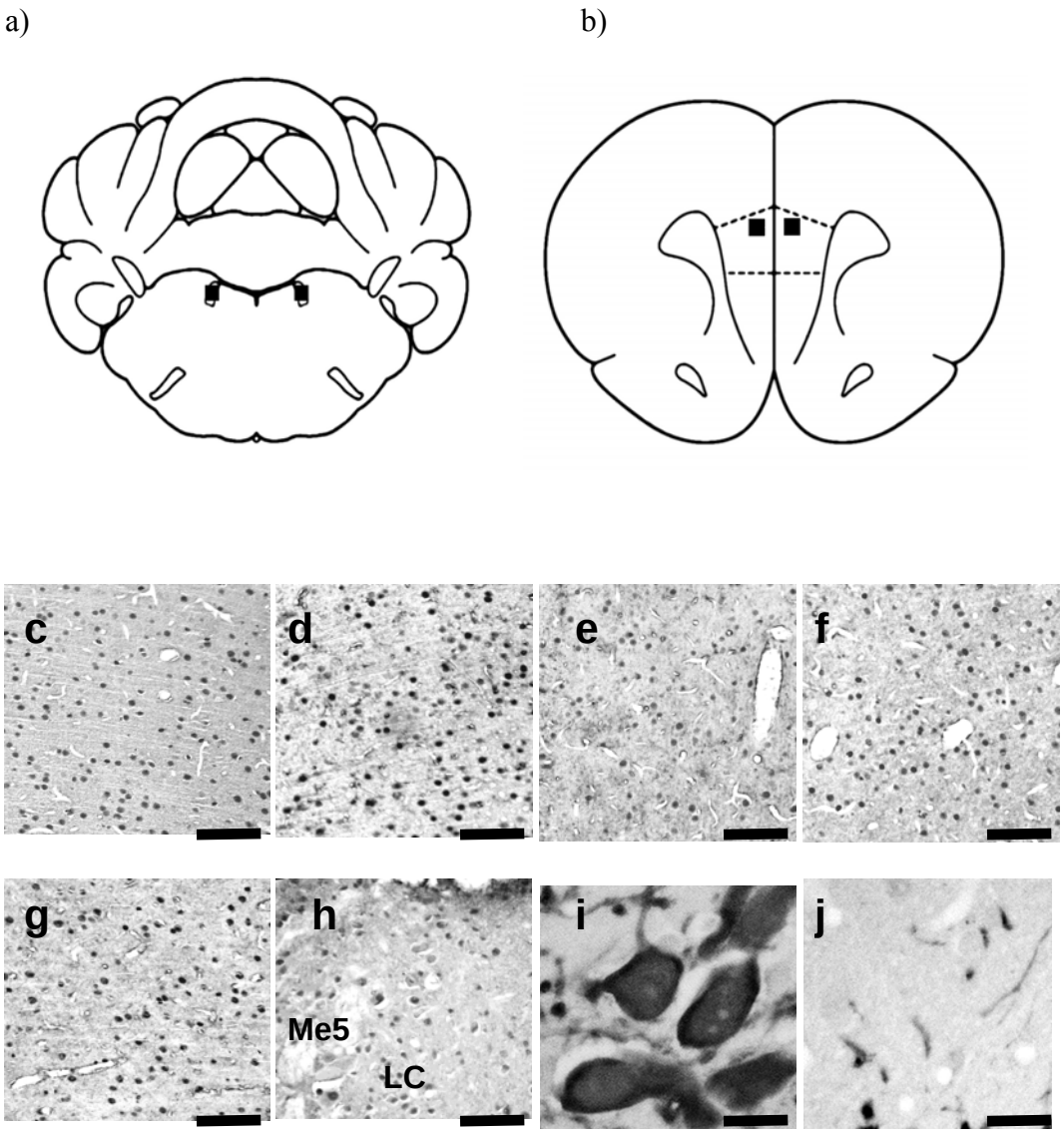


Fig. 12: The brain regions within which IEG immunoreactive nuclei and TH immunoreactive profiles were counted in the (a) LC and (b) prelimbic area of the mPFC in the rat brain. The areas sampled are denoted by black squares. Drawings are adapted from Paxinos and Watson (1998). Examples of immunostaining for (c) Fos-like protein and (d) c-Jun in the mPFC following treatment with 4 mg/kg/day olanzapine for 2 weeks; (e) ATF-2, (f) Egr-1 and (g) Egr-2 in the mPFC, (h) ATF-2 in the LC, and TH in the (i) LC and (j) mPFC following administration of acidified saline vehicle for 2 weeks. Scale bars:100 μm (c) to (g), (h) 150μm, and (i) and (j) 10 μm.

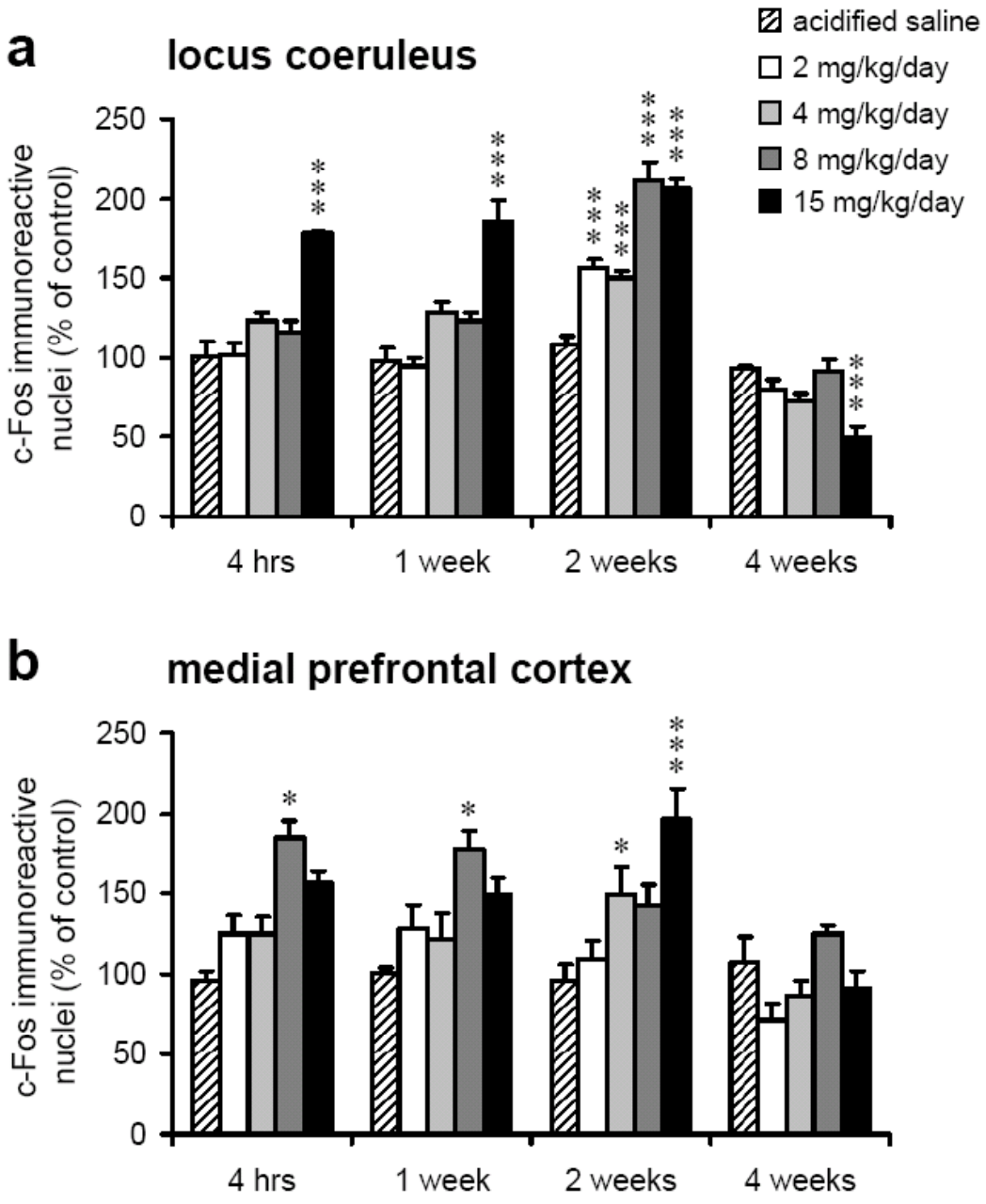


Fig. 13: Effects of olanzapine dose and treatment duration on c-Fos immunoreactivity in (a) the locus coeruleus (LC) and (b) the medial prefrontal cortex (mPFC). c-Fos immunoreactivity is expressed as the number of c-Fos immunopositive nuclei as a percentage of the pooled mean of the acidified saline vehicle control groups (mean ± sem). Following two-way analysis of variance, effects of olanzapine dose were compared with the acidified saline controls within each treatment duration by Tukey's HSD post hoc tests (* $p < 0.05$, *** $p < 0.0005$).

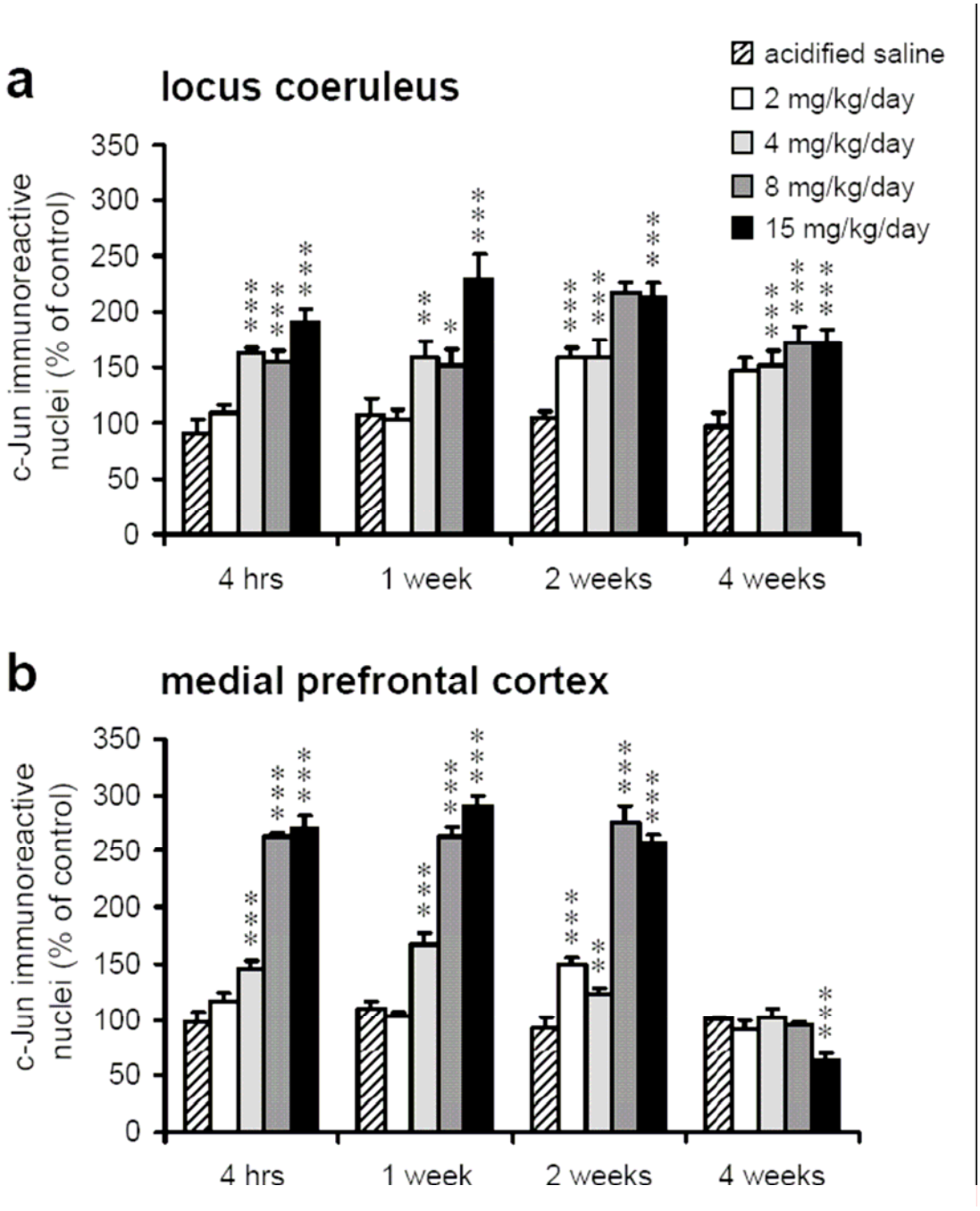


Fig. 14: Effects of olanzapine dose and treatment duration on c-Jun immunoreactivity in (a) the locus coeruleus (LC) and (b) the medial prefrontal cortex (mPFC). c-Jun immunoreactivity is expressed as the number of c-Jun immunopositive nuclei as a percentage of the pooled mean of the acidified saline vehicle control groups (mean ± sem). Following two-way analysis of variance, effects of olanzapine dose were compared with the acidified saline controls within each treatment duration by Tukey's HSD post hoc tests (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$).

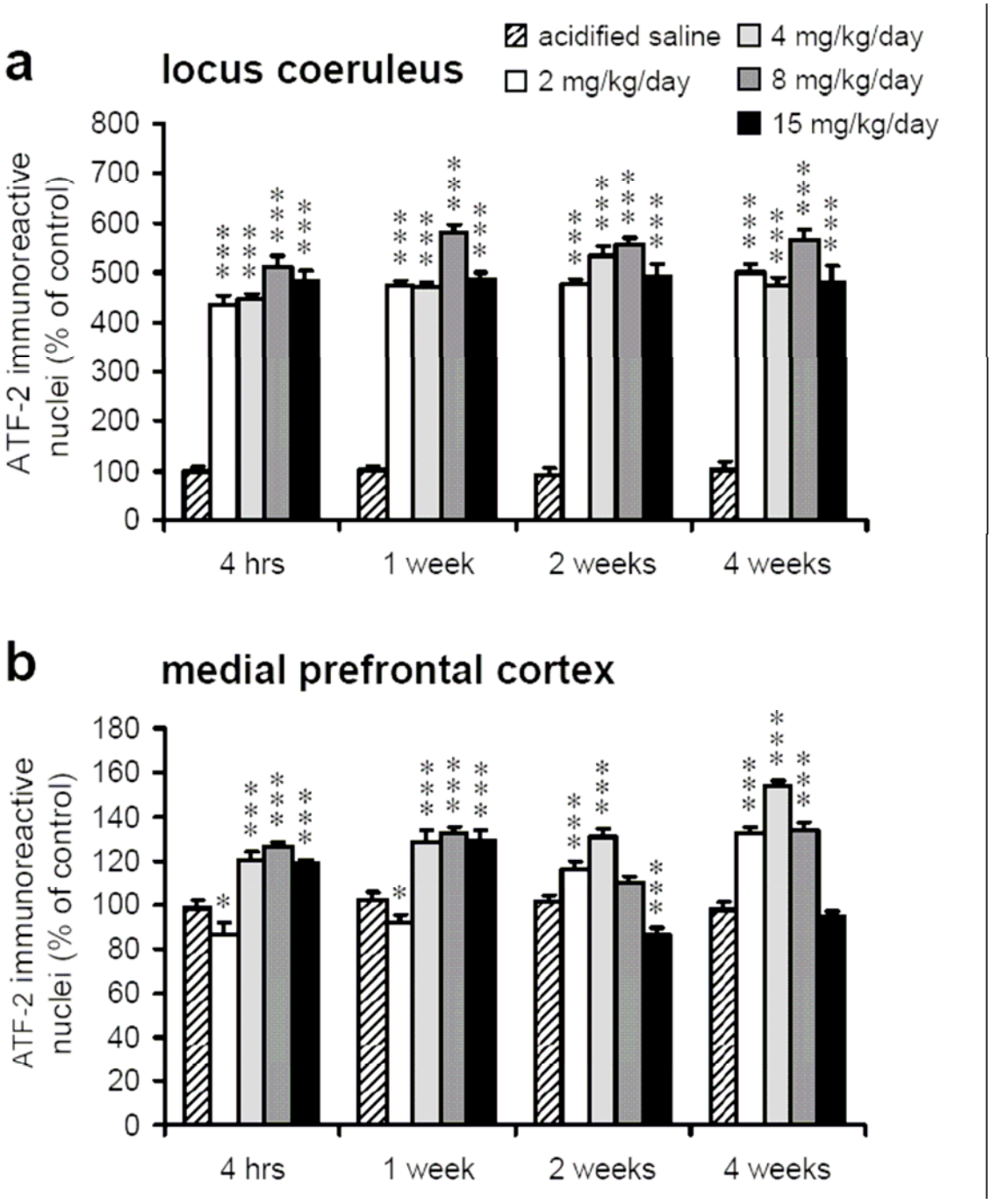


Fig. 15: Effects of olanzapine dose and treatment duration on ATF-2 immunoreactivity in (a) the locus coeruleus (LC) and (b) the medial prefrontal cortex (mPFC). ATF-2 immunoreactivity is expressed as the number of ATF-2 immunopositive nuclei as a percentage of the pooled mean of the acidified saline vehicle control groups (mean ± sem). Following two-way analysis of variance, effects of olanzapine dose were compared with the acidified saline controls within each treatment duration by Tukey's HSD post hoc tests (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$).

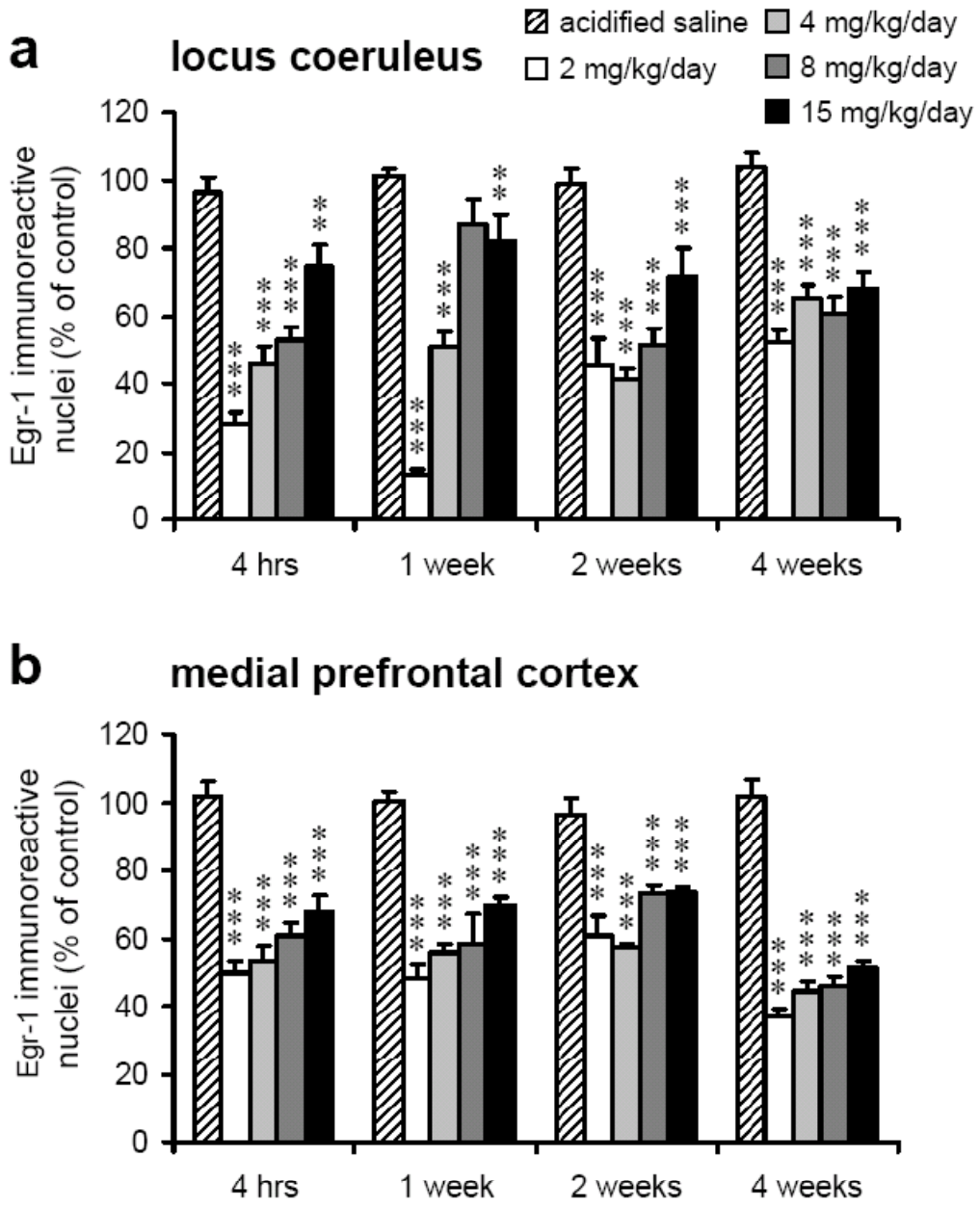


Fig. 16: Effects of olanzapine dose and treatment duration on Egr-1 immunoreactivity in (a) the locus coeruleus (LC) and (b) the medial prefrontal cortex (mPFC). Egr-1 immunoreactivity is expressed as the number of Egr-1 immunopositive nuclei as a percentage of the pooled mean of the acidified saline vehicle control groups (mean ± sem). Following two-way analysis of variance, effects of olanzapine dose were compared with the acidified saline controls within each treatment duration by Tukey's HSD post hoc tests (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$).

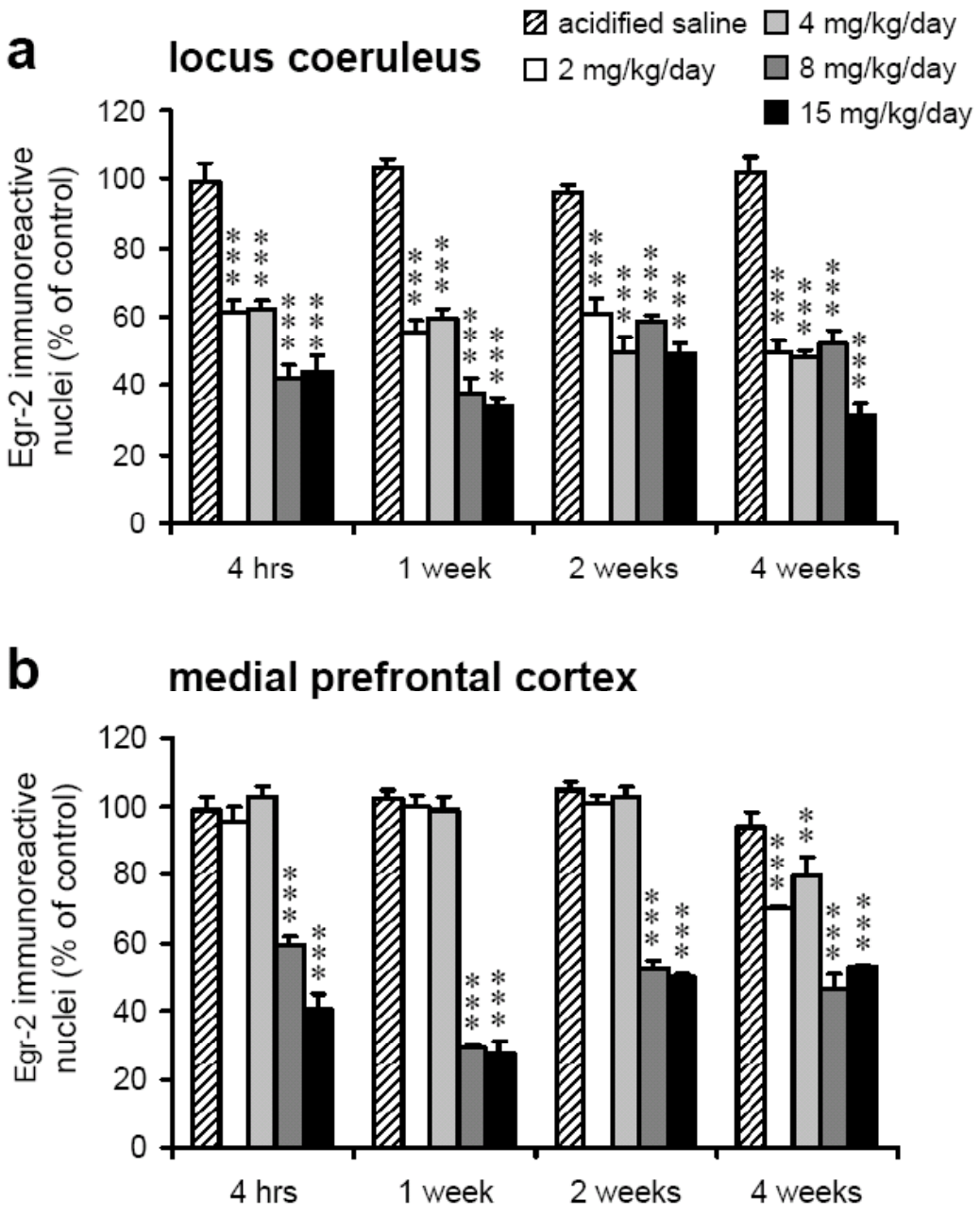


Fig. 17: Effects of olanzapine dose and treatment duration on Egr-2 immunoreactivity in (a) the locus coeruleus (LC) and (b) the medial prefrontal cortex (mPFC). Egr-2 immunoreactivity is expressed as the number of Egr-2 immunopositive nuclei as a percentage of the pooled mean of the acidified saline vehicle control groups (mean ± sem). Following two-way analysis of variance, effects of olanzapine dose were compared with the acidified saline controls within each treatment duration by Tukey's HSD post hoc tests (** $p < 0.005$, *** $p < 0.0005$).

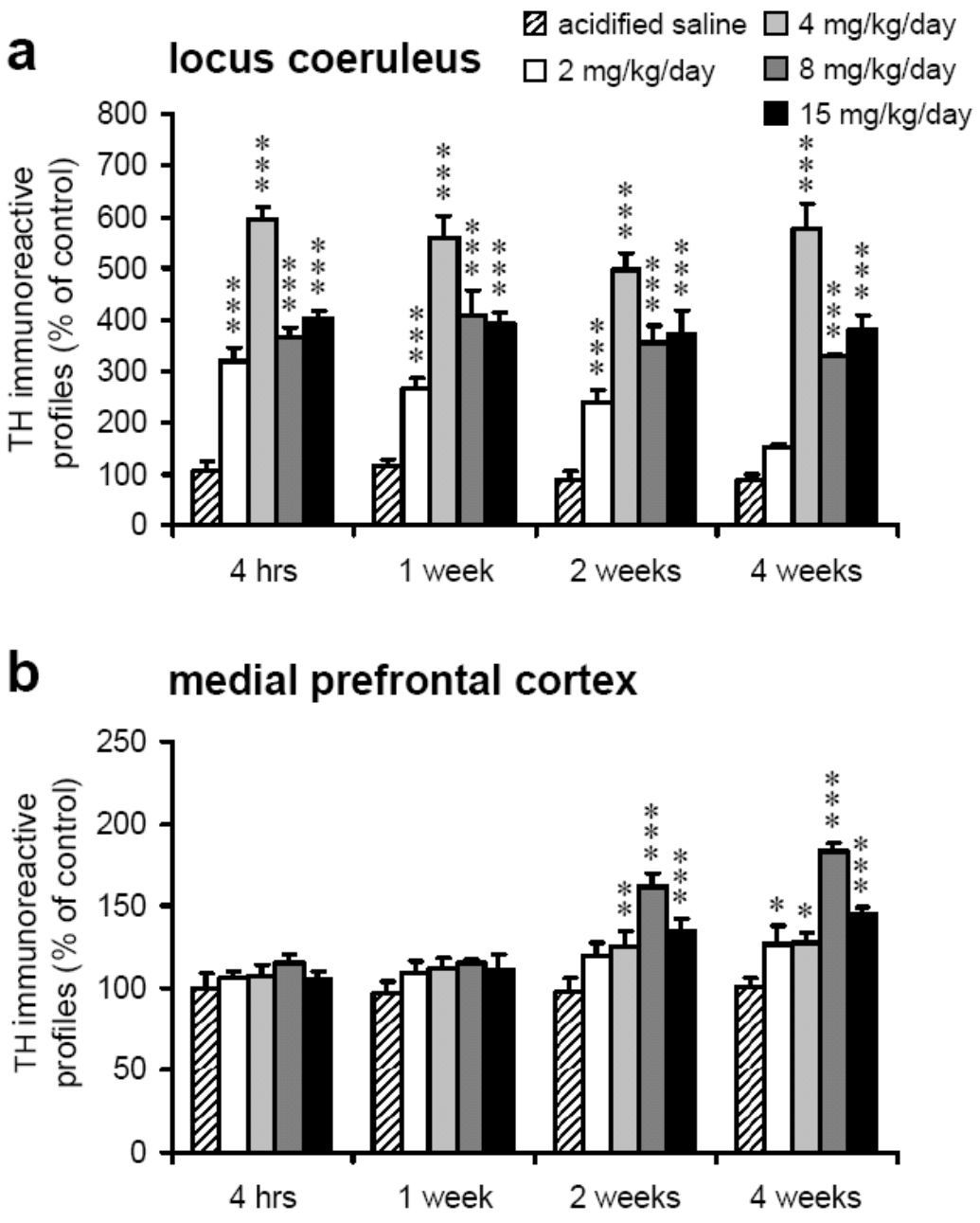


Fig. 18: Effects of olanzapine dose and treatment duration on tyrosine hydroxylase (TH) immunoreactivity in (a) the locus coeruleus (LC) and (b) the medial prefrontal cortex (mPFC). TH immunoreactivity is expressed as the number of TH immunopositive nuclei as a percentage of the pooled mean of the acidified saline vehicle control groups (mean ± sem). Following two-way analysis of variance, effects of olanzapine dose were compared with the acidified saline controls within each treatment duration by Tukey's HSD post hoc tests (** $p < 0.005$, *** $p < 0.0005$).

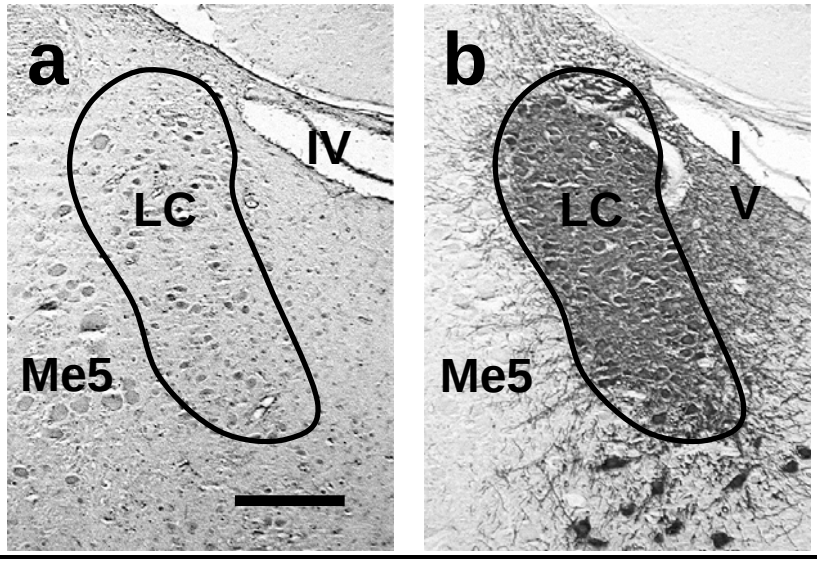


Fig.19: Representative photomicrographs of serial sections through the LC immunostained for (a) Egr-1 and (b) TH after administration of acidified saline for 2 weeks. IV, IVth ventricle; Me5, mesencephalic nucleus of the Vth nerve. The scale bar: 200 μm.

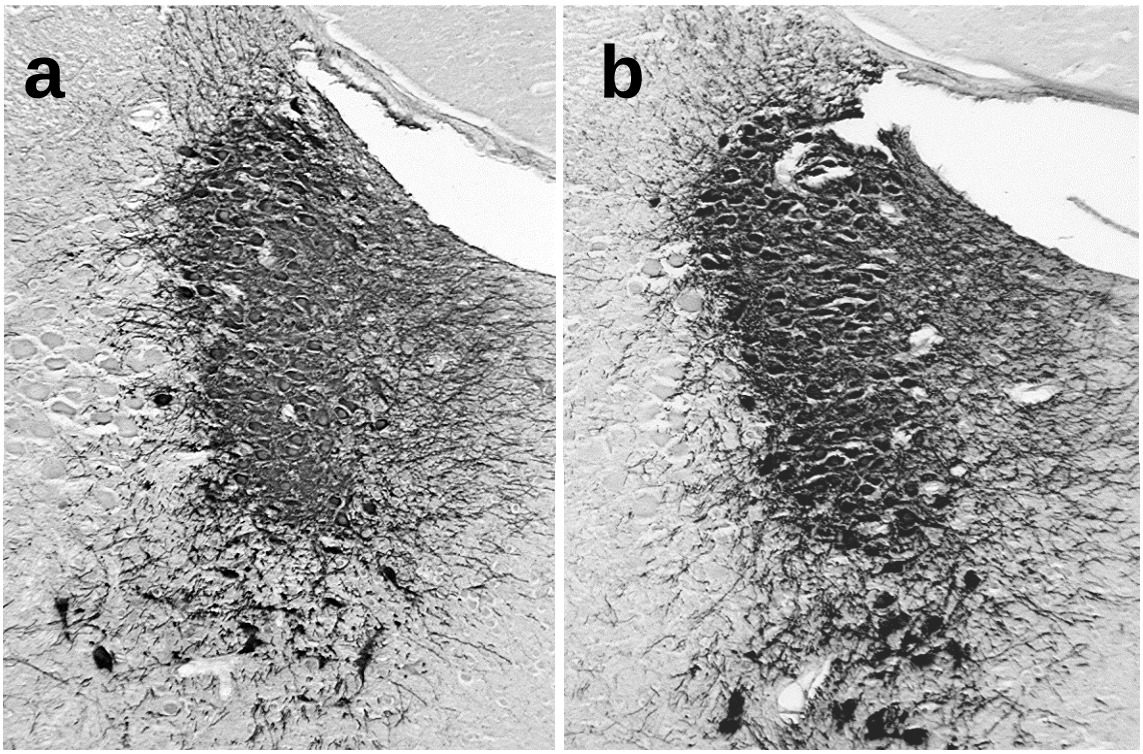


Fig. 20: Representative photomicrographs of TH immunoreactivity in the LC after administration of (a) acidified saline and (b) 8 mg/kg/day olanzapine for 1 week.. The scale bar: 200 μm.

3.2 *CHAKRAGATI* MOUSE EXPERIMENTS

3.2.1 Validity Experiments

A.) PPI Experiment: I started off with a routine PPI test comparing the wild type, heterozygous and the homozygous groups. Statistical analysis on a Two-factor ANOVA was done with genotype as between subjects factor and the trial type (prepulse intensity) as a repeated measure. The tests were significant for the genotype effect and the prepulse effect while the prepulse x genotype interaction effect was not significant. The results are as follows: prepulse effect $F_{(2,42)} = 30.91$, $p < 0.001$; genotype effect $F_{(2,21)} = 32.36$, $p < 0.001$; prepulse x genotype interaction effect $F_{(4,42)} = 0.416$, $p = 0.796$. Post hoc tests were carried out using Tukey's test. P level was set at 0.05. There was a significant reduction in the PPI of homozygous group. (**Figure. 21**)

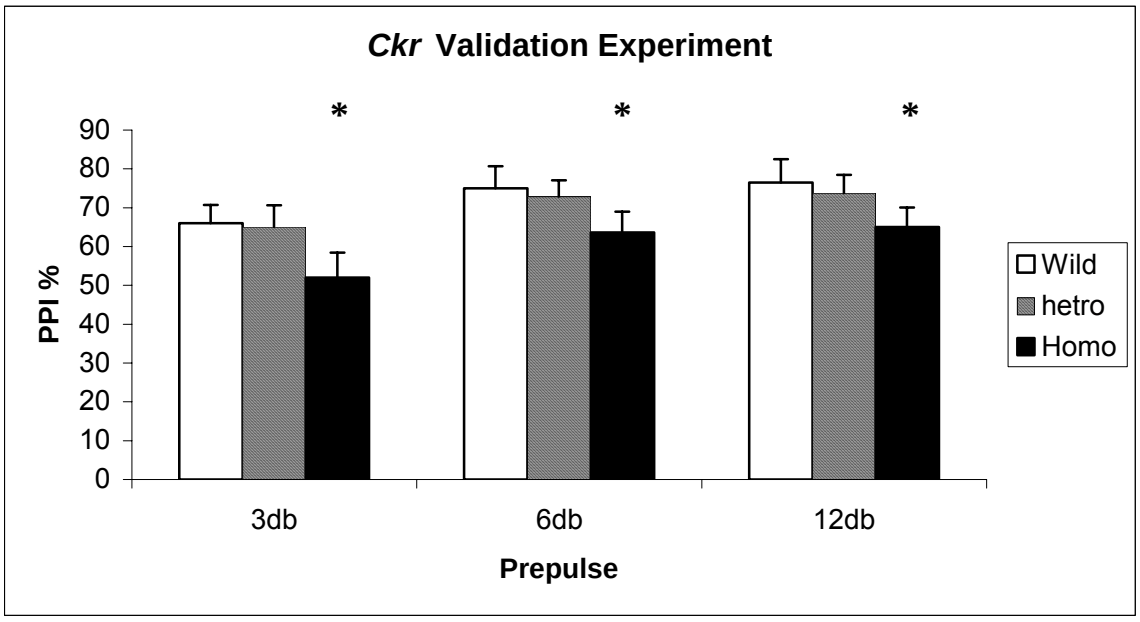


Fig. 21: Effects of gene manipulation on the Prepulse Inhibition in experimental mice (wild type, heterozygous and homozygous). The effect is expressed as prepulse inhibition percentage. Each point represents the mean ± SEM for these percentages. Following a Two-factor ANOVA with genotype as between subjects factor and the trial type (prepulse intensity) as a repeated measure, effects of gene manipulation in heterozygous and homozygous mice were compared with the wild type control mice for

each prepulse intensity category by Tukey's HSD post hoc tests.(* signifies $p < 0.05$ in comparison to the wild type group.).

The startle amplitude of all the three strains was also compared. Startle magnitude was analyzed with a one way ANOVA. Although the homozygous mice showed slightly raised startle amplitudes (266.87 ± 51.53) compared to the wild-type (221.62 ± 31.86) and the heterozygous strain (218.75 ± 50.25), this was not significant. (**Figure. 22**)

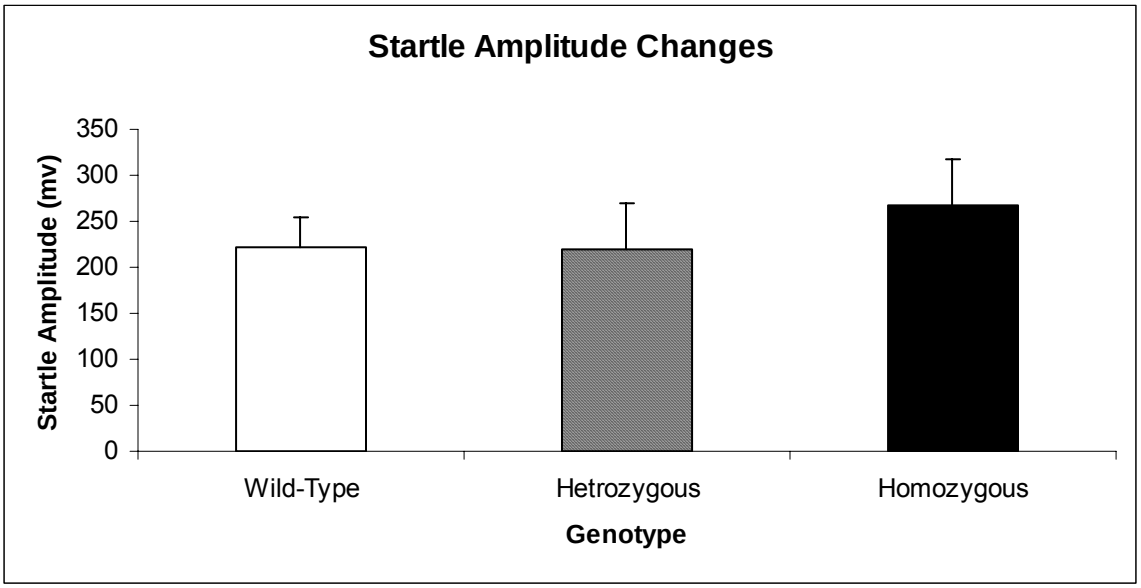


Fig. 22: Effects of gene manipulation on the startle amplitude in experimental mice (wild type, heterozygous and homozygous). The effect is expressed as the startle response in mille volts. The points represent the mean ± SEM for the pulse alone amplitudes

Following the routine PPI test I tried to ascertain if the mice were suffering from any hearing defect. For this we tested only the homozygous mice, but the time duration between the prepulse and pulse sounds were altered (25ms, 100 ms and 175 ms) with 100 ms being the control value. Statistical analysis was done for double repeated measures (time gaps and prepulse intensity) for the homozygous mice. The PPI were significantly lower when the time gap between prepulse and pulse was reduced to 25 ms. There was not much difference in PPI for time gaps 100 ms and 175 ms. The tests were significant for the time gap effect, prepulse intensity effect as well as time gap x prepulse intensity

interaction effect. The resuls are as follows: prepulse effect $F_{(2,14)} = 35.19$, $p < 0.001$; Time gap effect $F_{(2,14)} = 1158.15$, $p < 0.001$; prepulse x time gap interaction effect $F_{(4,28)} = 5.00$, $p = 0.004$. (**Figure. 23**)

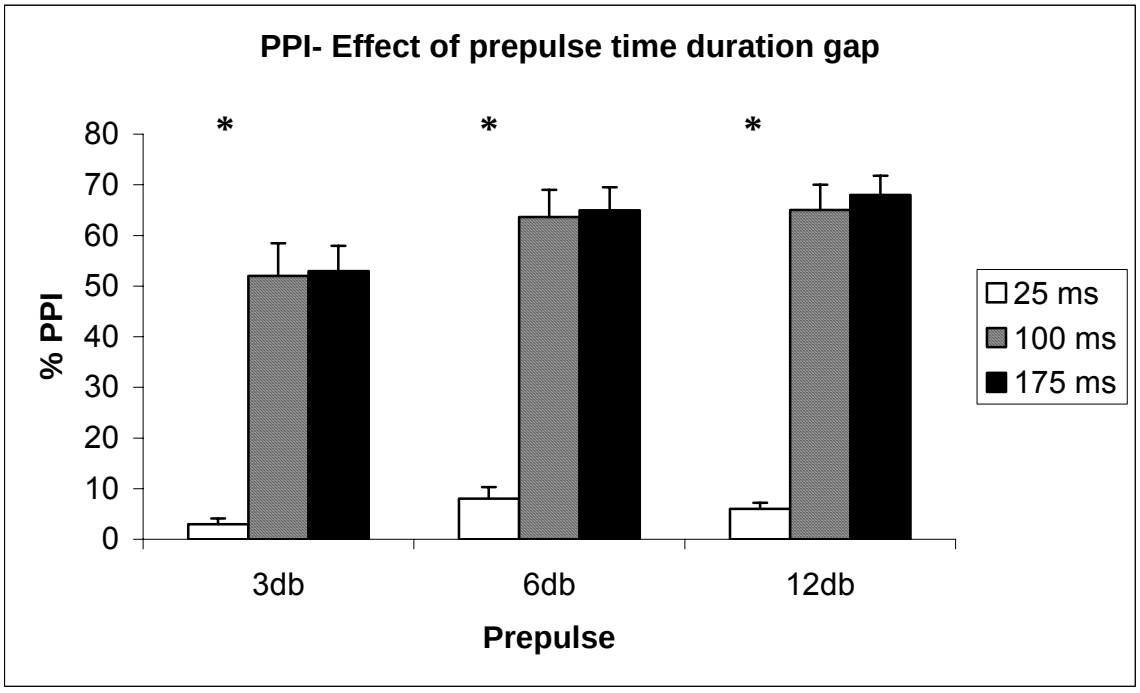
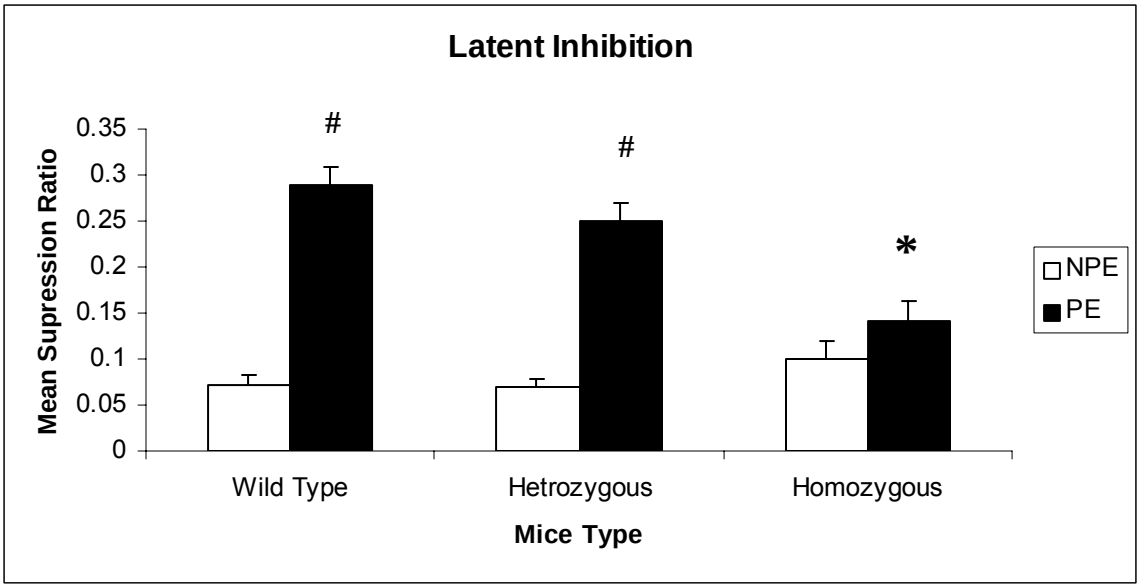


Fig. 23: Effects of different time gaps between prepulse and the pulse tones, on the Prepulse Inhibition in experimental mice (homozygous strain). The effect is expressed as prepulse inhibition percentage. Each point represents the mean ± SEM for these percentages. Statistical analysis was done for double repeated measures (time gaps and prepulse intensity) for the homozygous mice. Post hoc test was done usning Tukey's HSD test. (* signifies $p < 0.001$ in comparison to the 100ms group in the same prepulse category).

B.) LI Experiment: For the LI experiment statistical analysis was done using a Two-factor ANOVA (Exposure and genotype were the two fixed factors). The results showed a significant genotype effect, exposure effect as well as genotype x exposure interaction effect on the suppression rations. The results were as follows: genotype effect $F_{(2,18)} = 24.47$, $p < 0.001$; exposure effect $F_{(1,18)} = 411.46$, $p < 0.001$; exposure x genotype interaction effect $F_{(2,18)} = 55.09$, $p < 0.001$. Post hoc tests were done using the Tukey's test and it was seen that the pre-exposed homozygous mice had significantly lower

suppression ratio as compared to the pre-exposed witld-type and pre-exposed heterozygous mice. Also there was not significantly different suppression ratio for exposed and non-pre exposed animals in the homozygous category. This result was different from the wild-type and the heterozygous groups which showed significantly different suppression ratios between their respective pre-exposed and non pre-exposed animals. (**Figure. 24**)



NPE = Non Pre-Exposure; PE = Pre-Exposure

Fig. 24: Effects of gene manipulation on the Latent Inhibition in experimental mice (wild type, heterozygous and homozygous). The effect is expressed as suppression ratio. Each point represents the mean ± SEM for these ratios. Following a two-way analysis of varience, effects of gene manipulation in heterozygous and homozygous mice were compared with the wild type control mice for each exposure category by Tukey's HSD post hoc tests. (* signifies $p < 0.001$ in comparison to the wild-type pre-exposed group. # signifies $p < 0.001$ in comparison to the non pre-exposed animals of the same genotype).

3.2.2 Drug Experiments

A.) **Phase I:** It was found out that irrespective of the treatment given to the wild type mice, there was no significant change in their PPI percentage as compared to the baseline PPI for the wild type. The baseline PPI of the homozygous mice was significantly lower than the baseline PPI of the wild type mice ($p< 0.01$). Administration of risperidone or haloperidol did not lead to any significant change in the PPI of the homozygous mice, while administration of clozapine (all three dosages) led to a significant increase in the PPI of the homozygous mice as compared to their baseline values. Statistical analysis was done using a two factor ANOVA (genotype and the drug treatment being the fixed factors). The results are as followings: treatment effect $F_{(9,140)} = 5.97$, $p < 0.001$; genotype effect $F_{(1,140)} = 186.61$, $p < 0.001$; genotype x treatment effect $F_{(9,140)} = 3.71$, $p < 0.001$. Post hoc test was utilized and it showed significantly higher PPI values for all the three clozapine treated homozygous groups compared to the baseline homozygous PPI (**Fig. 25**)

To analyze the data pertaining to the startle amplitudes, we conducted a one way ANOVA for the different treatment groups, both for wild type as well as homozygous strains. Risperidone (all doses), haloperidol (all doses) and the two lower doses of clozapine did not show any significant effect on the startle amplitudes. It was found out that for both wild type mice and the homozygous mice, the group receiving high dosage of clozapine had significantly lower startle amplitude than the control group. The result in the wild type mice was $F_{(9,70)} = 3.58$, $p = 0.001$. (**Figure. 26**). The result in the homozygous mice was $F_{(9,70)} = 2.55$, $p = 0.013$ (**Figure. 27**). Post hoc tests were done using Tukey's test.

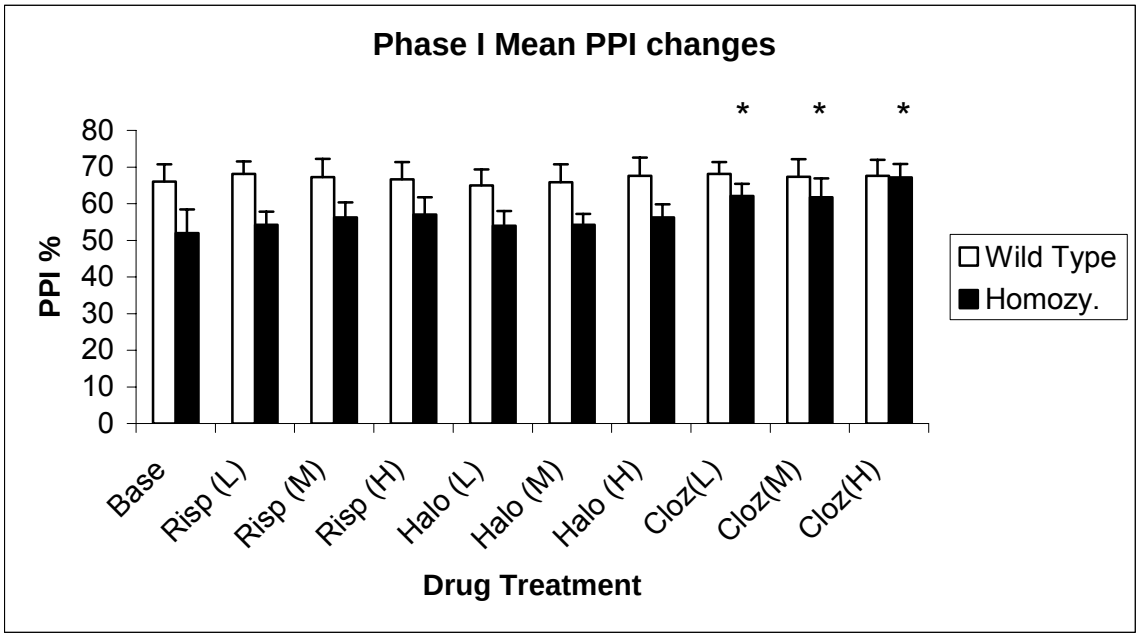


Fig. 25: Showing the effect of drug treatment on the PPI of wild type as well as the homozygous strain of *ckr* mice. * signifies $p < 0.05$ compared to the baseline homozygous PPI.

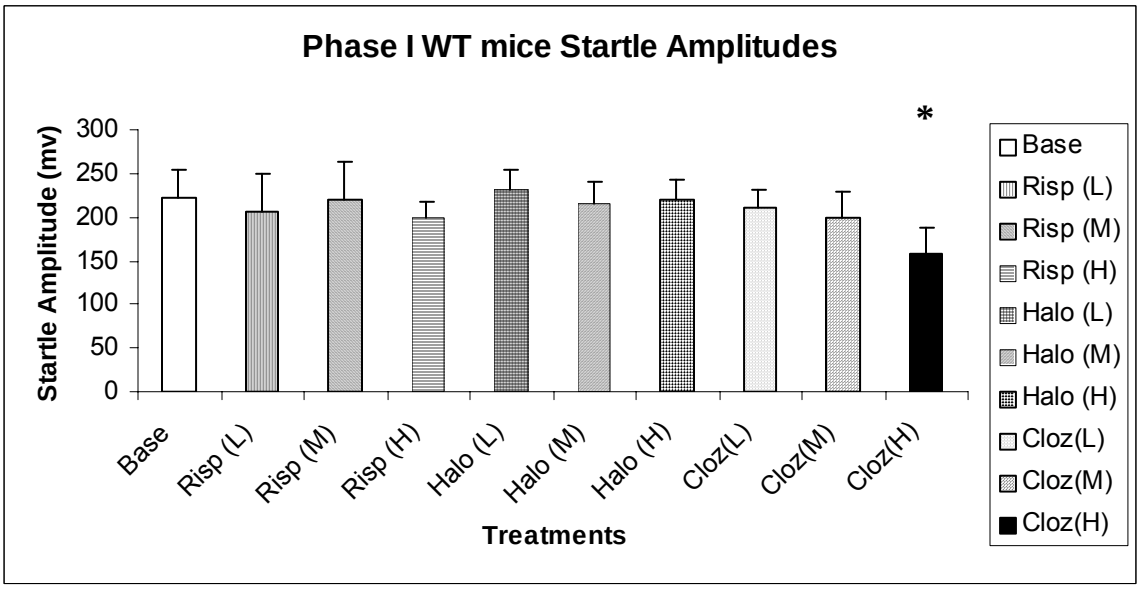


Fig. 26: Showing the effect of drug treatment on the startle amplitude of wild type mice. * signifies $p < 0.05$ compared to the baseline startle amplitude of the wild type control group.

Treatments: 1) Base = No drug treatment; 2) Risp (L) = Risperidone 0.1 mg/kg ; 3) Risp (M) = Risperidone 0.5 mg/kg; 4) Risp (H) = Risperidone 1.0 mg/kg; 5) Halo (L) = Haloperidol 0.1 mg/kg; 6) Halo (M) = Haloperidol 0.5 mg/kg; 7) Halo (H) = Haloperidol 1.0 mg/kg; 8) Cloz(L) = Clozapine 1.0 mg/kg; 9) Cloz(M) = Clozapine 4.0 mg/kg; 10) Cloz(H) = Clozapine 10.0 mg/kg

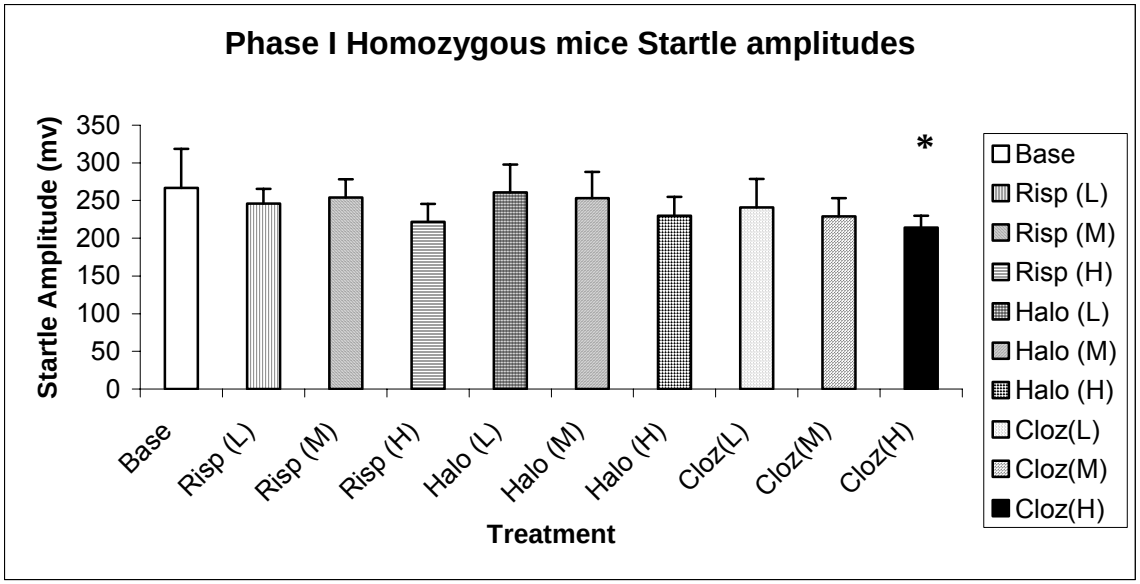


Fig. 27: Showing the effect of drug treatment on the startle amplitude of homozygous *ckr* mice. * signifies $p < 0.05$ compared to the baseline startle amplitude of the homozygous control group.

Treatments: 1) Base = No drug treatment; **2)** Risp (L) = Risperidone 0.1 mg/kg ; **3)** Risp (M) = Risperidone 0.5 mg/kg; **4)** Risp (H) = Risperidone 1.0 mg/kg; **5)** Halo (L) = Haloperidol 0.1 mg/kg; **6)** Halo (M) = Haloperidol 0.5 mg/kg; **7)** Halo (H) = Haloperidol 1.0 mg/kg; **8)** Cloz(L) = Clozapine 1.0 mg/kg; **9)** Cloz(M) = Clozapine 4.0 mg/kg; **10)** Cloz(H) = Clozapine 10.0 mg/kg

B.) Phase II: In phase II experiments also, the various drug treatments failed to produce any significant change in the PPI percentage of the wild type mice. For the homozygous mice, all the treatments showed an increase in the PPI %, except for alpha1 agonist drug. Statistical analysis was done using a two-factor ANOVA (genotype and the drug treatment being the fixed factors). The effect of genotype, treatment and genotype x treatment interaction were all significant. The results are as followings: treatment effect $F_{(6,98)} = 7.15$, $p < 0.001$; genotype effect $F_{(1,98)} = 5.59$, $p = 0.020$; genotype x treatment effect $F_{(6,98)} = 7.16$, $p < 0.001$. Post hoc tests were done using Tukey's test and all the three dose combinations of clozapine and prazosin were found to be having significantly higher PPI than the baseline PPI in homozygous mice. (**Figure. 28**)

To analyze the data pertaining to the startle amplitudes, we conducted a one-way ANOVA for the different treatment groups, both for wild type as well as homozygous strains. Except for two treatment groups (High Dose Clozapine ; High dose clozapine + alpha-1 antagonist), no other treatment group showed any significant effect on the startle amplitude. It was found out that for both wild type mice and the homozygous mice, the group receiving high dosage of clozapine and the group receiving the combination of clozapine (high dosage) and alpha1-antagonist, had significantly lower startle amplitude than the control group. The result in the wild type mice was $F_{(6,49)} = 10.05$, $p < 0.001$ (**Figure. 29**). The result in the homozygous mice was $F_{(6,49)} = 8.06$, $p < 0.001$ (**Figure. 30**). Post hoc tests were done using Tukey's test.

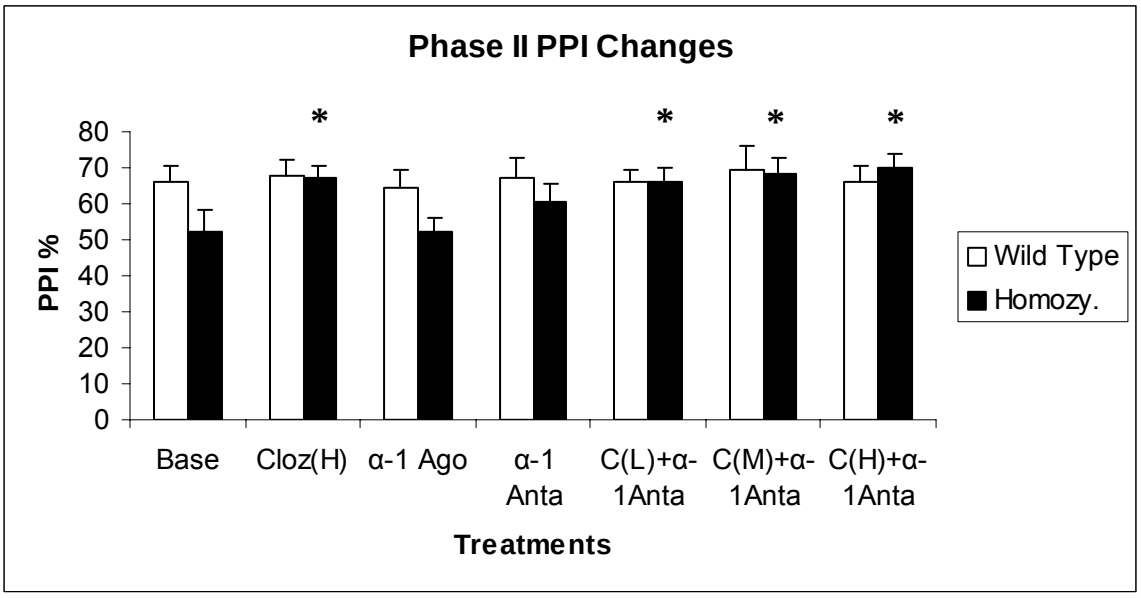


Fig. 28: Showing the effect of drug treatment on the PPI of wild type as well as the homozygous strain of chakra mice. * $p < 0.05$ compared to the baseline homozygous PPI. [Note: the data for baseline control group and Clozapine (high dosage) group is the same as in phase I study]

Treatments: 1) Base = No drug treatment; **2)** Cloz (H) = Clozapine 10 mg/kg/day ; **3)** α-1 Ago = Cirazoline 0.75 mg/kg/day; **4)** α-1 Anta = Prazosin 1.0 mg/kg/day; **5)** C(L) + α-1 Anta = Clozapine 1.0 mg/kg/day + Prazosin 1.0 mg/kg/day; **6)** C(M) + α-1 Anta = Clozapine 4.0 mg/kg/day + Prazosin 1.0 mg/kg/day; **7)** C(H) + α-1 Anta = Clozapine 10.0 mg/kg/day + Prazosin 1.0 mg/kg/day

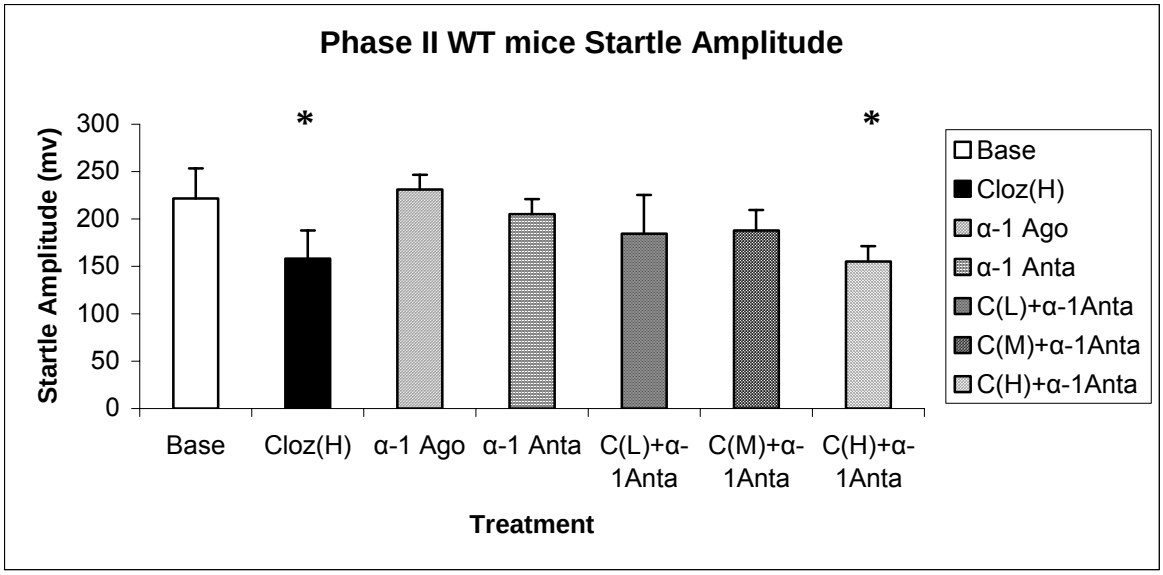


Fig. 29: Showing the effect of drug treatment on the startle amplitude of wild type mice. * signifies $p < 0.05$ compared to the startle amplitude of baseline control wild type mice. [Note: the data for baseline control group and Clozapine (high dosage) group is the same as in phase I study]

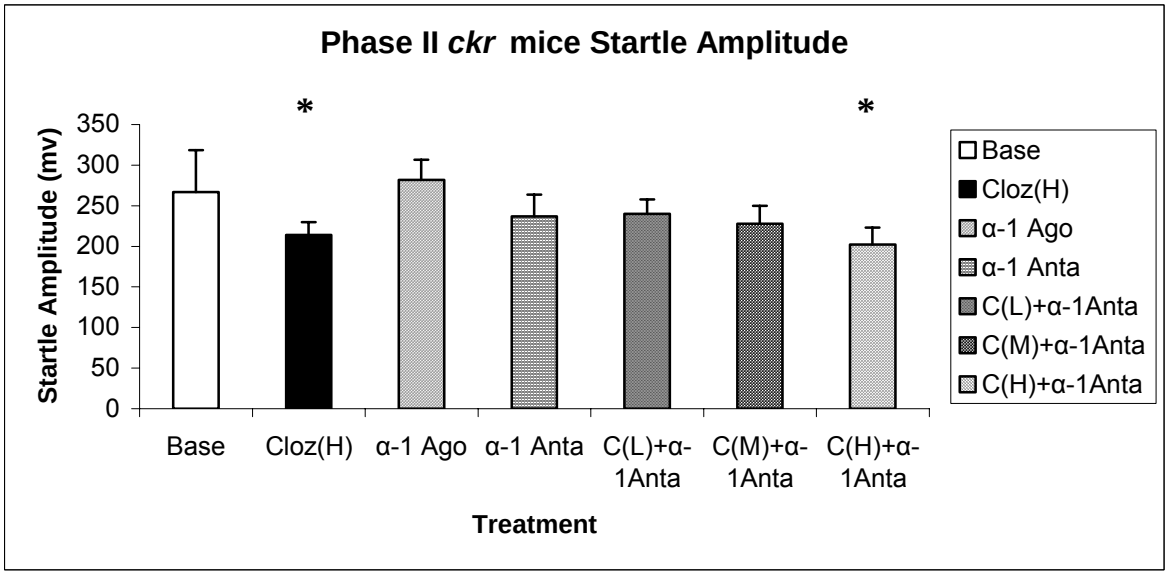


Fig. 30: Showing the effect of drug treatment on the startle amplitude of homozygous *ckr* mice. * signifies $p < 0.05$ compared to the baseline startle amplitude of the control homozygous mice. [Note: the data for baseline control group and Clozapine (high dosage) group is the same as in phase I study]

Treatments: 1) Base = No drug treatment; **2)** Cloz (H) = Clozapine 10 mg/kg/day ; **3)** α-1 Ago = Cirazline 0.75 mg/kg/day; **4)** α-1 Anta = Prazosin 1.0 mg/kg/day; **5)** C(L) + α-1 Anta = Clozapine 1.0 mg/kg/day + Prazosin 1.0 mg/kg/day; **6)** C(M) + α-1 Anta = Clozapine 4.0 mg/kg/day + Prazosin 1.0 mg/kg/day; **7)** C(H) + α-1 Anta = Clozapine 10.0 mg/kg/day + Prazosin 1.0 mg/kg/day

3.3 RAT EXPERIMENTS

3.3.1 Water maze experiments

A) Phase I: Latencies, swim distances and the swim speeds required to locate the hidden platform in the water maze for days 1-4 are shown in the figures below.

Analysis showed that the rats treated with MK-801 had significantly higher latencies as compared to the control group ($p<0.01$). Addition of haloperidol to MK-801 did not improve the situation. But when olanzapine was added to MK-801, the combination had significantly lesser latencies than ones treated with MK-801 alone ($p < 0.001$). There was significant improvement seen and MK-801 + olanzapine group showed latencies which were more comparable to the control group. Olanzapine had led to the reversal of the effect of MK-801. Statistical comparisons for latencies across the four groups revealed the following results: treatment effect $F_{(3,28)} = 273.53$, $p< 0.001$; day effect $F_{(3,84)} = 79.94$, $p < 0.001$; day x treatment interaction $F_{(9,84)} = 24.87$, $p < 0.001$. For post hoc, Tukey HSD test was done (**Figure 31**).

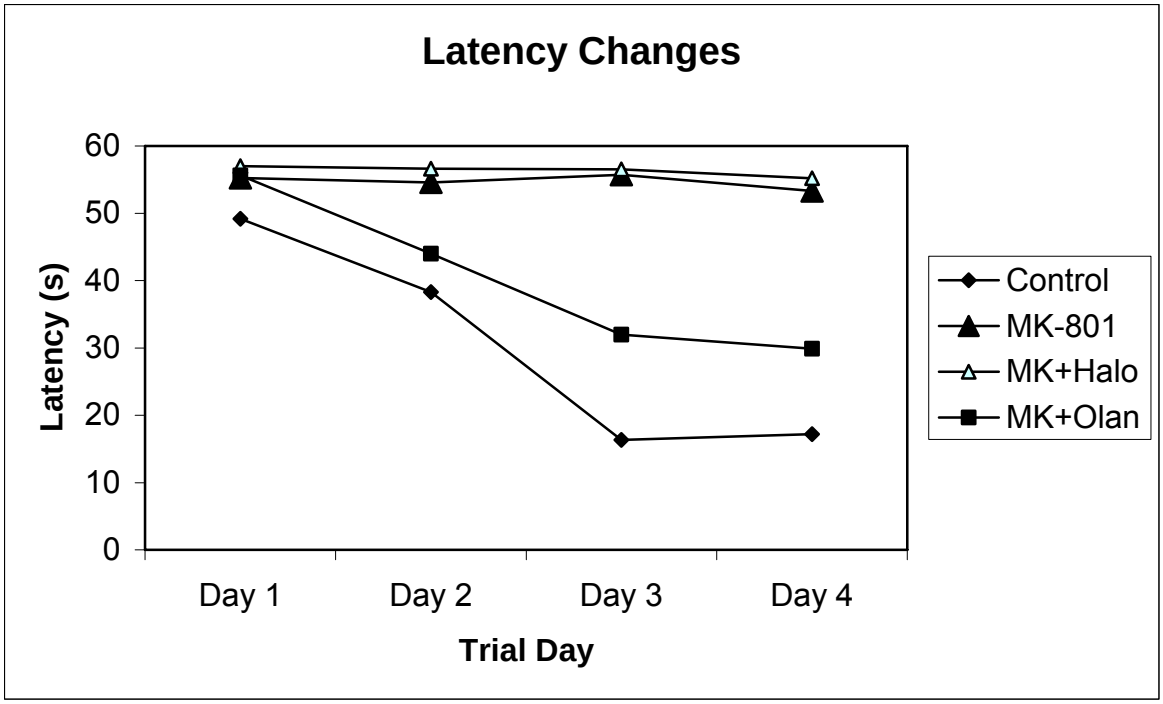


Fig. 31: Effects of chronic exposure to different drug treatments on latency to find a hidden platform in a water maze task on 4 consecutive days of testing compared to vehicle controls.

Treatments: 1) Base + Control group; **2)** MK-801 = 0.1 mg/kg on the day of the experiment; **3)** MK + Halo = Haloperidol 0.5 mg/kg/day for 4 weeks + MK-801 0.1 mg/kg on day of experiment; **4)** MK + Olan = Olanzapine 10 mg/kg/day for 4 weeks + MK-801 0.1 mg/kg on day of experiment

Analysis of the swim distances showed that treatment with MK-801 led to significant increase in the swim distance as compared to the control group ($p< 0.01$). Addition of olanzapine to MK-801, did improve the situation by bringing the swim distances down but these were not significant enough. The rats treated in the control group and the group treated with the combination of MK-801 and Haloperidol showed significantly shorter distances traveled as compared to the MK-801 group ($p < 0.001$). Statistical comparisons for distances across the four groups revealed the following results: treatment effect $F_{(3,28)} = 34.64$, $p< 0.001$; day effect $F_{(3,84)} = 19.7$, $p < 0.001$; day x treatment interaction $F_{(9,84)} = 11.7$, $p < 0.001$. For post hoc, Tukey HSD test was conducted (**Figure 32**).

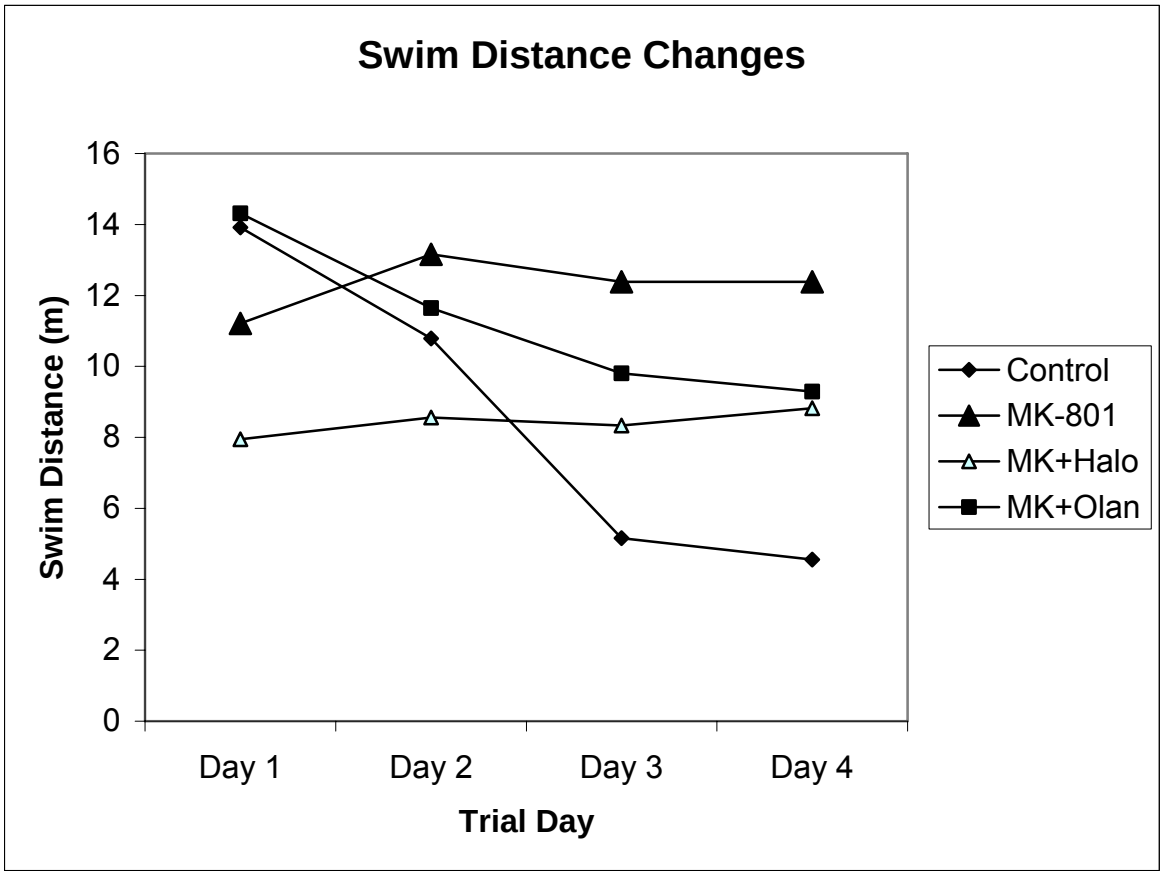


Fig. 32: Effects of chronic exposure to different drug treatments on the swim distance to find a hidden platform in a water maze task on 4 consecutive days of testing compared to vehicle controls.

Treatments: 1) Base + Control group; **2)** MK-801 = 0.1 mg/kg on the day of the experiment; **3)** MK + Halo = Haloperidol 0.5 mg/kg/day for 4 weeks + MK-801 0.1 mg/kg on day of experiment; **4)** MK + Olan = Olanzapine 10 mg/kg/day for 4 weeks + MK-801 0.1 mg/kg on day of experiment

Analysis for swim speeds showed that the MK-801 + Haloperidol grouphad the slowest swim speed. MK-801 alone also decreased the swim speeds as compared to the control group. But addition of olanzapine to MK-801 led to the reversal of this and these rats showed significantly faster swim speed compared to the MK-801 alone treated group ($p < 0.001$). Statistical comparisons for swim speeds across the four groups revealed the

following results: treatment effect $F(3,28) = 87.4$, $p < 0.001$; day effect $F(3,84) = 6.86$, $p < 0.001$; day x treatment interaction $F(9,84) = 2.12$, $p < 0.05$. For post hoc, Tukeys HSD tests were conducted. (**Figure 33**)

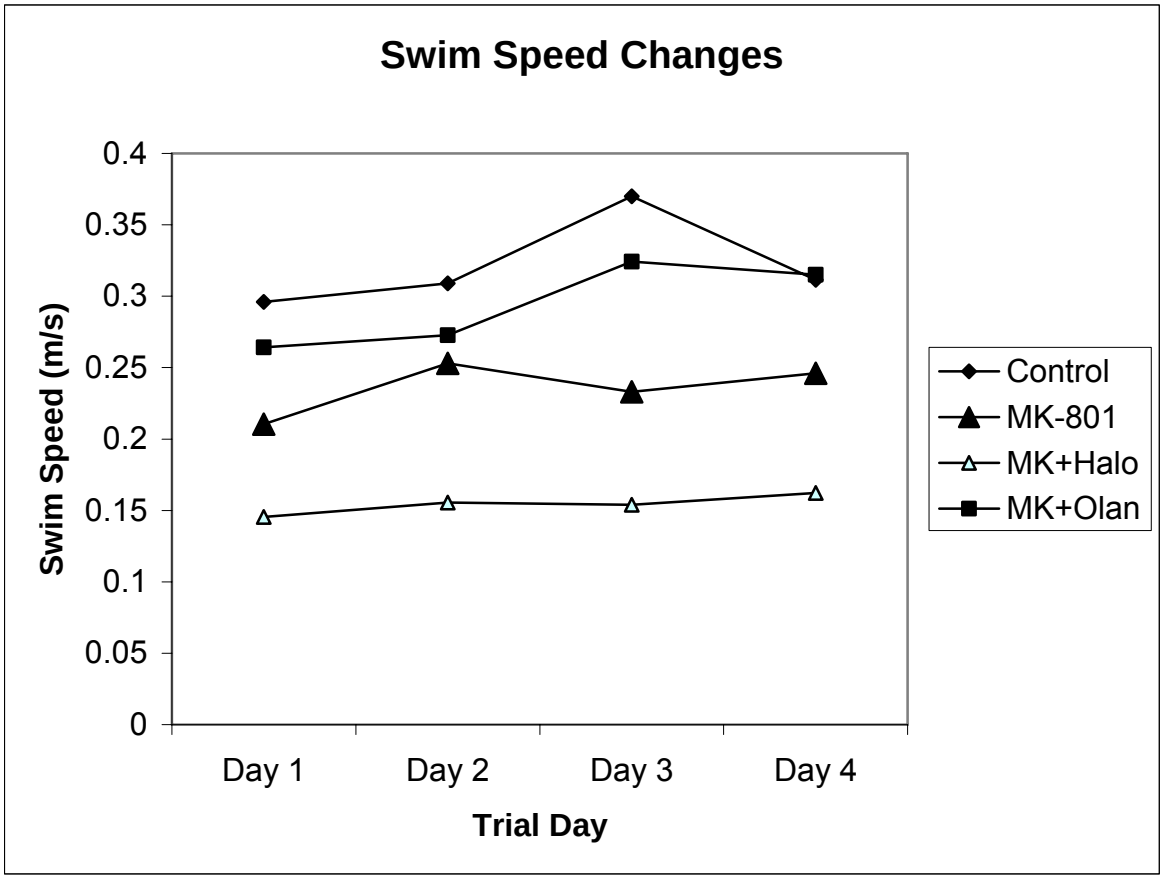


Fig. 33: Effects of chronic exposure to different drug treatments on swim speed while trying to find a hidden platform in a water maze task on 4 consecutive days of testing compared to vehicle controls.

Treatments: 1) Base + Control group; **2)** MK-801 = 0.1 mg/kg on the day of the experiment; **3)** MK + Halo = Haloperidol 0.5 mg/kg/day for 4 weeks + MK-801 0.1 mg/kg on day of experiment; **4)** MK + Olan = Olanzapine 10 mg/kg/day for 4 weeks + MK-801 0.1 mg/kg on day of experiment

B) Phase II experiment: Latencies, swim distances and the swim speeds required to locate the hidden platform in the water maze for days 1-4 are shown in the figures below.

The following treatment groups were studied:

1) MK-801 = MK-801 0.1 mg/kg on the day of the experiment;

2) MK + Olan = Olanzapine 10 mg/kg/day for 4 weeks + MK-801 0.1 mg/kg on day of experiment

3) Group A = Prazosin 1.0 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment

4) Group B = Cirazoline 0.75 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment

5) Group C = Idazoxan 1.5 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment

6) Group D = Clonidine 0.2 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment;

Statistical comparisons for latencies across the six groups revealed the following results: treatment effect $F_{(5,42)} = 81.49$, $p < 0.001$; day effect $F_{(3,126)} = 155.2$, $p < 0.001$; day x treatment interaction $F_{(15,126)} = 18.26$, $p < 0.001$. Post hoc Tukey HSD test showed that the rats in group C (containing alpha-2 antagonist idazoxan), had significantly shorter latencies than the ones treated with MK-801 + Olanzapine combination ($p < 0.001$). (**Figure 34**)

Statistical comparisons for swim distances across the six groups revealed the following results: treatment effect $F_{(5,42)} = 14.87$, $p < 0.001$; day effect $F_{(3,126)} = 44.50$, $p < 0.001$; day x treatment interaction $F_{(15,126)} = 12.99$, $p < 0.001$. Post hoc Tukeys HSD test showed that the rats in group D (containing alpha-2 agonist clonidine), had significantly longer

swim distances than the ones treated with MK-801+ Olanzapine combination ($p < 0.001$). (**Figure 35**)

Statistical comparisons for swim speed across the six groups revealed the following results: treatment effect $F_{(5,42)} = 23.79$, $p< 0.001$; day effect $F_{(3,126)} = 6.71$, $p < 0.001$. While these two were statistically significant, the day x treatment interaction did not show any significance $F_{(15,126)} = 1.70$, $p = 0.058$. Post hoc Tukey HSD test showed that the rats in group C (containing alpha-2 antagonist idazoxan), had significantly faster swim speeds than the ones treated with MK-801+Olanzapine combination ($p < 0.05$). (**Figure 36**)

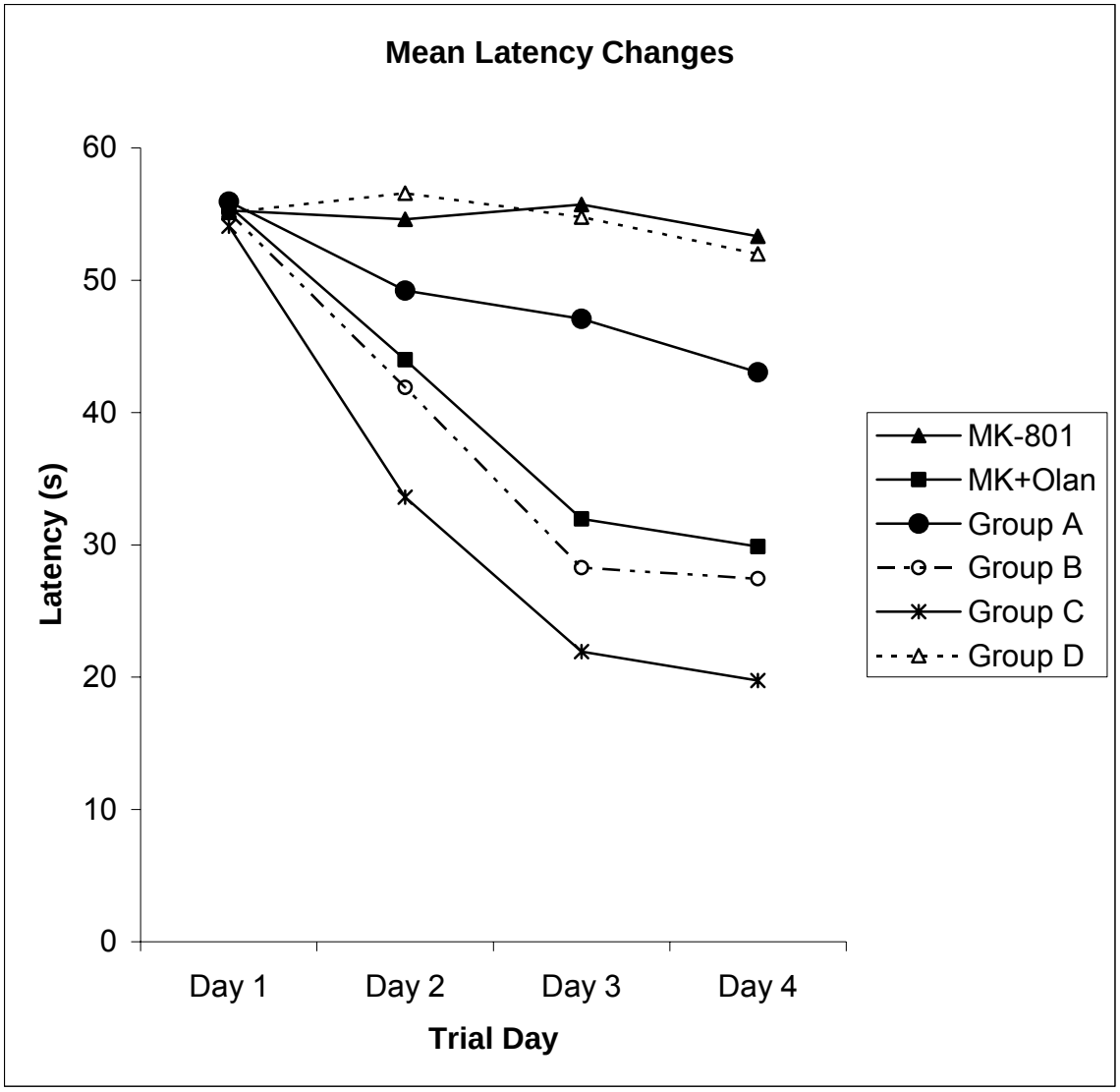


Fig. 34: Effects of chronic exposure to different drug treatments on latency while trying to find a hidden platform in a water maze task on 4 consecutive days of testing.

Treatments: 1) MK-801 = 0.1 mg/kg on the day of the experiment; **2)** MK + Olan = Olanzapine 10 mg/kg/day for 4 weeks + MK-801 0.1 mg/kg on day of experiment; **3)** Group A = Prazosin 1.0 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment; **4)** Group B = Cirazoline 0.75 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment; **5)** Group C = Idazoxan 1.5 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment; **6)** Group D = Clonidine 0.2 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment;

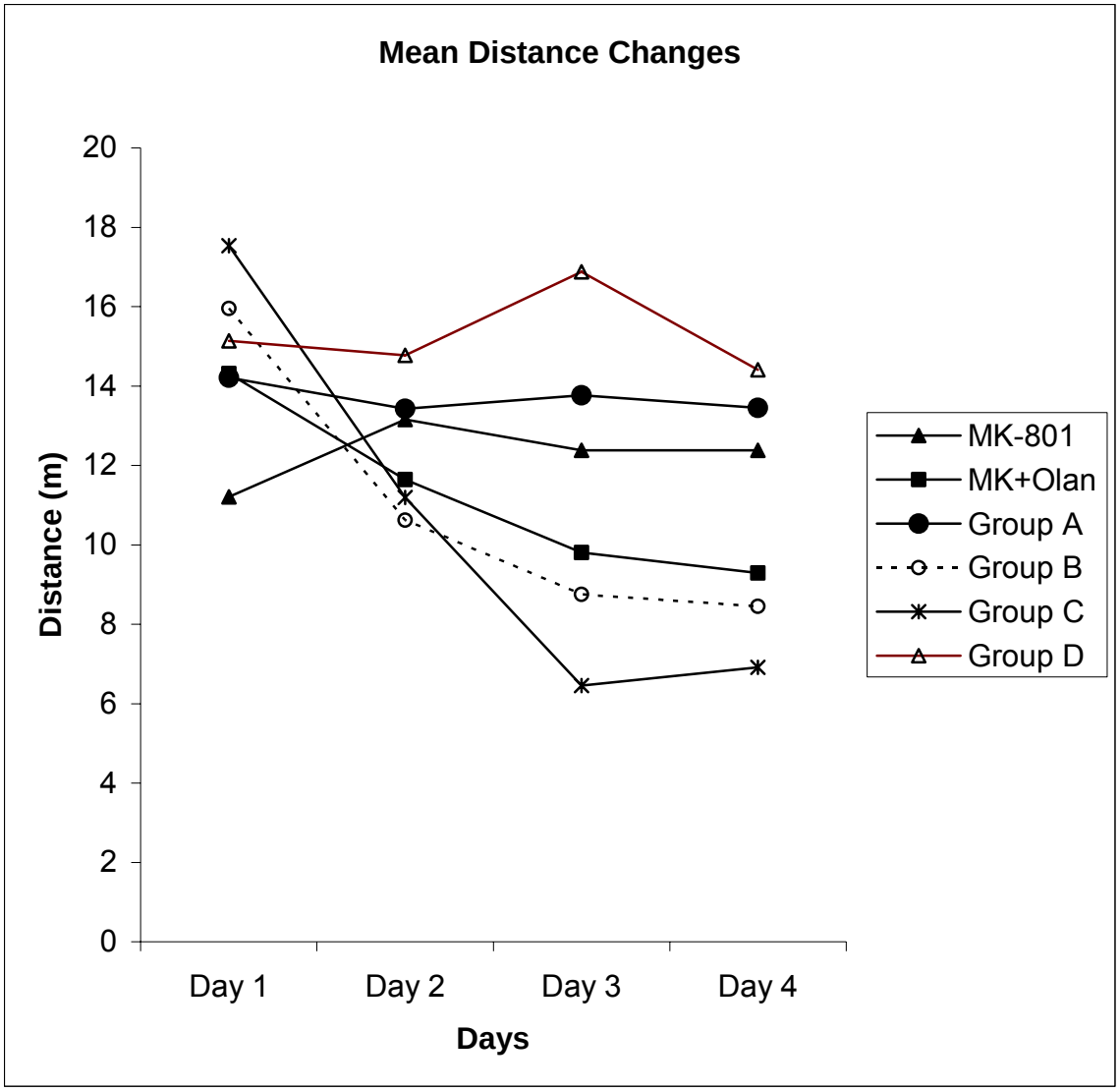


Fig. 35: Effects of chronic exposure to different drug treatments on swim distance while trying to find a hidden platform in a water maze task on 4 consecutive days of testing.

Treatments: 1) MK-801 = 0.1 mg/kg on the day of the experiment; **2)** MK + Olan = Olanzapine 10 mg/kg/day for 4 weeks + MK-801 0.1 mg/kg on day of experiment; **3)** Group A = Prazosin 1.0 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment; **4)** Group B = Cirazoline 0.75 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment; **5)** Group C = Idazoxan 1.5 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment; **6)** Group D = Clonidine 0.2 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment;

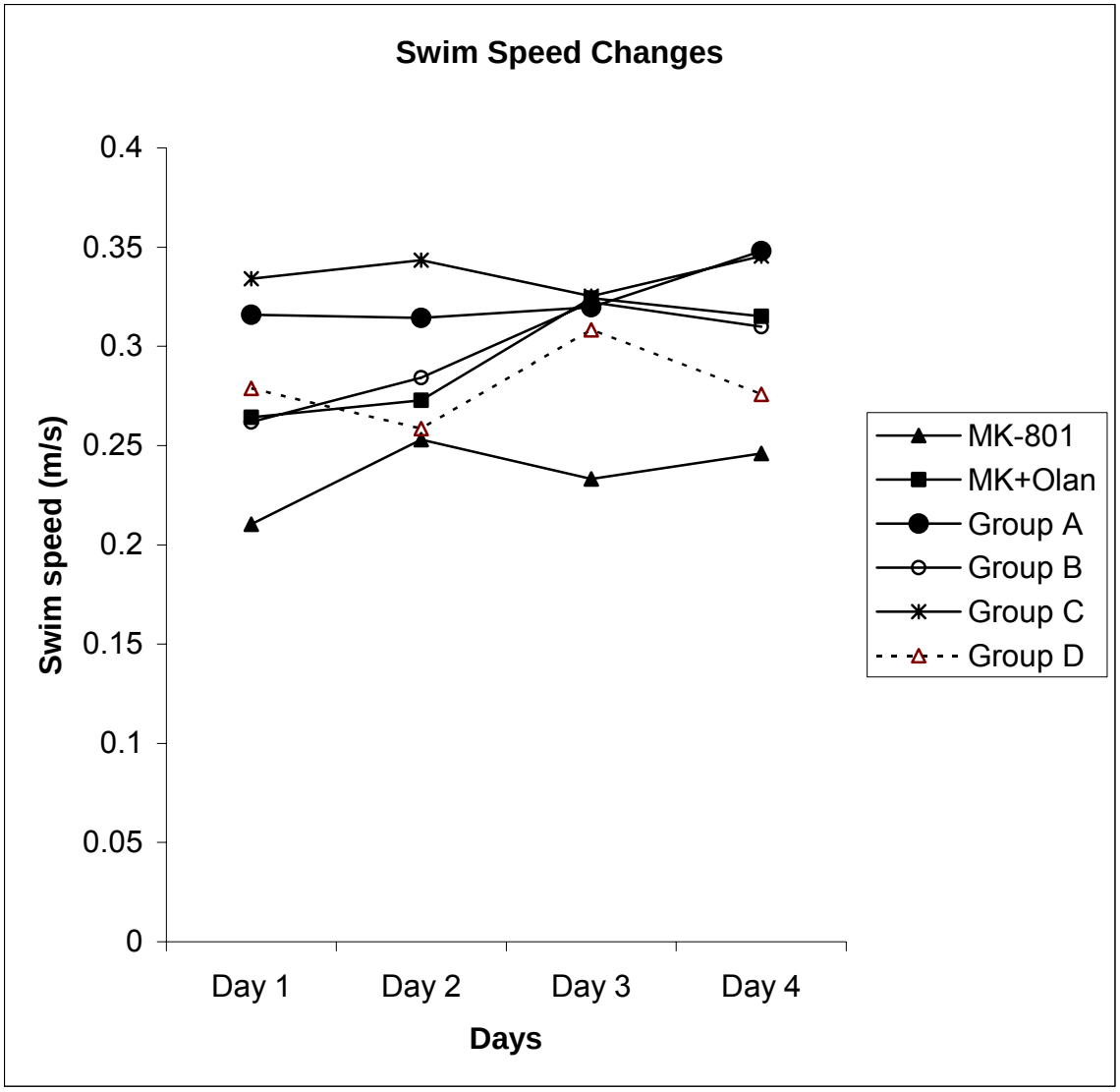


Fig. 36: Effects of chronic exposure to different drug treatments on swim speed while trying to find a hidden platform in a water maze task on 4 consecutive days of testing.

Treatments: 1) MK-801 = 0.1 mg/kg on the day of the experiment; **2)** MK + Olan = Olanzapine 10 mg/kg/day for 4 weeks + MK-801 0.1 mg/kg on day of experiment; **3)** Group A = Prazosin 1.0 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment; **4)** Group B = Cirazoline 0.75 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment; **5)** Group C = Idazoxan 1.5 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment; **6)** Group D = Clonidine 0.2 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment

3.3.2 PPI Experiments

A) Phase I : The results show that compared to the control animals, the treatment with MK-801 leads to a marked deficit in the PPI. This effect is seen to be reversed by haloperidol as well as olanzapine treatments. The turnaround is more so in case of olanzapine. Statistical analysis following a two way ANOVA (Time-duration and the treatment being the two fixed factors) show significant effect of treatment while the treatment duration (weeks) or the weeks x treatment interaction, both failed to show any significance. The results are the following: treatment effect $F_{(3,28)} = 15214.7$, $p < 0.001$; treatment duration (week) effect $F_{(2,56)} = 1.05$, $p = 0.355$; Week x Treatment effect $F_{(6,56)} = 1.65$, $p = 0.151$. Post hoc tests showed that the Mk-801 + olanzapine treatment did significantly better than the MK-801 alone group and also the MK-801 + haloperidol group. (**Figure 37**)

A two-way ANOVA was done to analyze the startle amplitudes of the different groups with drug treatment as between groups factor and weeks of treatment as repeated measures. There was no effect of treatment duration on the startle amplitudes. Only the drug treatment had significant effect on the startle amplitude. MK-801 treated group had significantly higher startle amplitude than the control group at 1 week and 2 week treatment durations while this effect was not seen at 4 week treatment duration. Addition of olanzapine to MK-801, helped in bringing down the startle amplitudes.This effect was significant compared to the MK-801 alone treatment group at 2 weeks of treatment duration. The results were as follows: Week effect $F_{(2,56)} = 0.280$, $p = 0.757$; treatment

effect $F_{(3,28)} = 10.49$, $p < 0.001$; week x treatment effect $F_{(6,56)} = 0.882$, $p = 0.514$. Post hoc test were conducted using Tukey's test . (**Figure 38**)

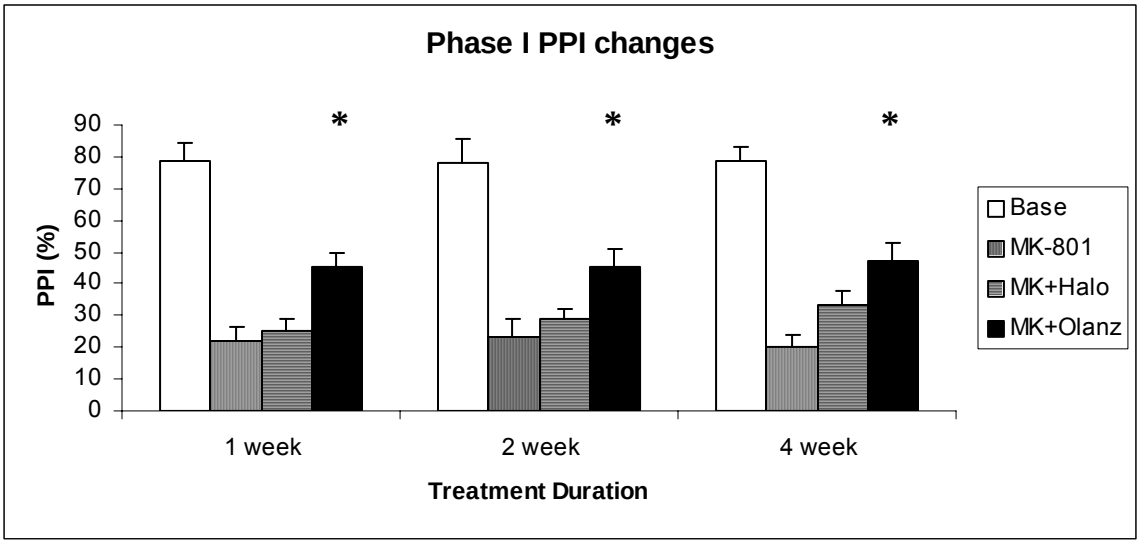


Fig. 37: Showing the effect of various drug treatments on the prepulse inhibition in rats. Each point signifies the PPPI (mean ± SEM) for that group. For post hoc, Tukey's test was conducted (* $p < 0.001$ when compared to MK-801 treatment group)

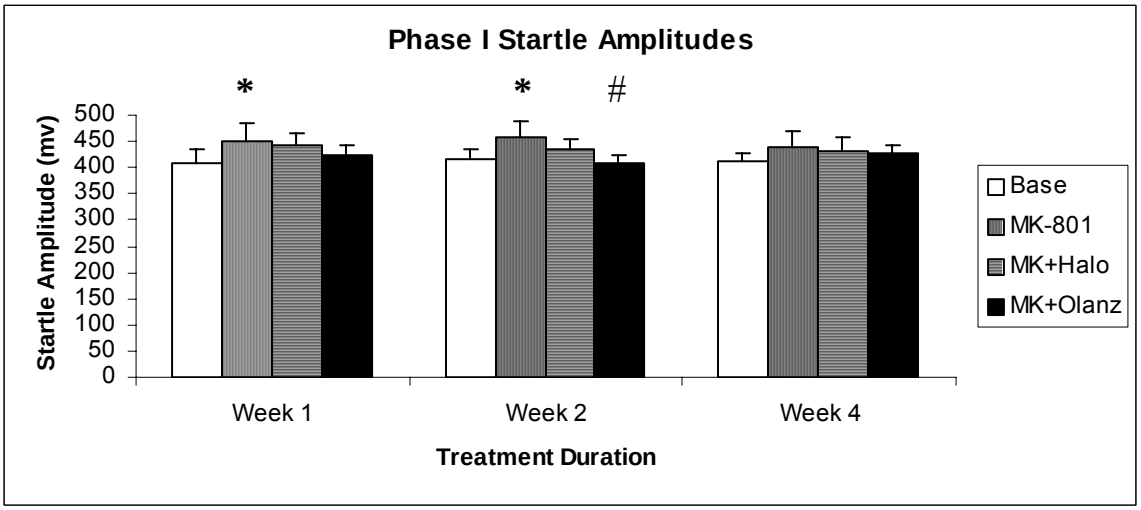


Fig. 38: Showing the effect of various drug treatments on the startle amplitude in rats. Each point signifies the Amplitude (mean ± SEM) for that group. For post hoc, Tukey's test was conducted (* signifies $p < 0.05$ compared to the baseline control group for that duration of treatment; # signifies $p < 0.05$ compared to the MK-801 group for that treatment duration).

Treatments: 1) Base + Control group; **2)** MK-801 = 0.1 mg/kg on the day of the experiment; **3)** MK + Halo = Haloperidol 0.5 mg/kg/day for 4 weeks + MK-801 0.1 mg/kg on day of experiment; **4)** MK + Olan = Olanzapine 10 mg/kg/day for 4 weeks + MK-801 0.1 mg/kg on day of experiment

B) Phase II: The results show that adding alpha1 antagonist prazosin to the M-801 + olanzapine combination significantly increases the attenuation of the prepulse inhibition deficit caused by MK-801. Other treatments with alpha 1 agonist, alpha2 agonist and alpha2 antagonist do not significantly change the results obtained with the MK-801 + olanzapine combination. Statistical analysis following a two way ANOVA (Time-duration and the treatment being the two fixed factors) show significant effect of treatment and the treatment duration (weeks) while the weeks x treatment interaction, failed to show any significance. The results are as following: treatment effect $F_{(5,42)}$ = 241.30, $p < 0.001$; treatment duration (week) effect $F_{(2,84)}$ = 3.62, p = 0.031; Week x Treatment effect $F_{(10,84)}$ = 1.0, p = 0.242. Post hoc tests showed that the [alpha1 antagonist prazosin + Olanzapine + MK-801] group had significantly better results than the [Olanzapine + MK-801] group. (**Figure 39**)

A two-way ANOVA was done to analyze the startle amplitudes of the different groups with drug treatment as between groups factor and weeks of treatment as repeated measures. The duration of treatment had no effect on the startle amplitudes. Only the drug treatment had significant effect on the startle amplitude. At 1 week treatment duration, Group C (containing alpha-1 antagonist Prazosin) showed significantly lower amplitude compared to the MK-801 group. At 2 weeks of treatment duration, group MK-801 + olanzapine group, group C and group D (containing alpha-2 agonist clonidine) showed significantly lower startle amplitudes compared to the MK-801 group. At 4 weeks there were no significant differences between the treatment groups. The results were as follows: Week effect $F_{(2,84)}$ = 1.971, p = 0.146; treatment effect $F_{(5,42)}$ = 17.14, $p < 0.001$; week x treatment effect $F_{(10,84)}$ = 1.596, p = 0.122. (**Figure 40**)

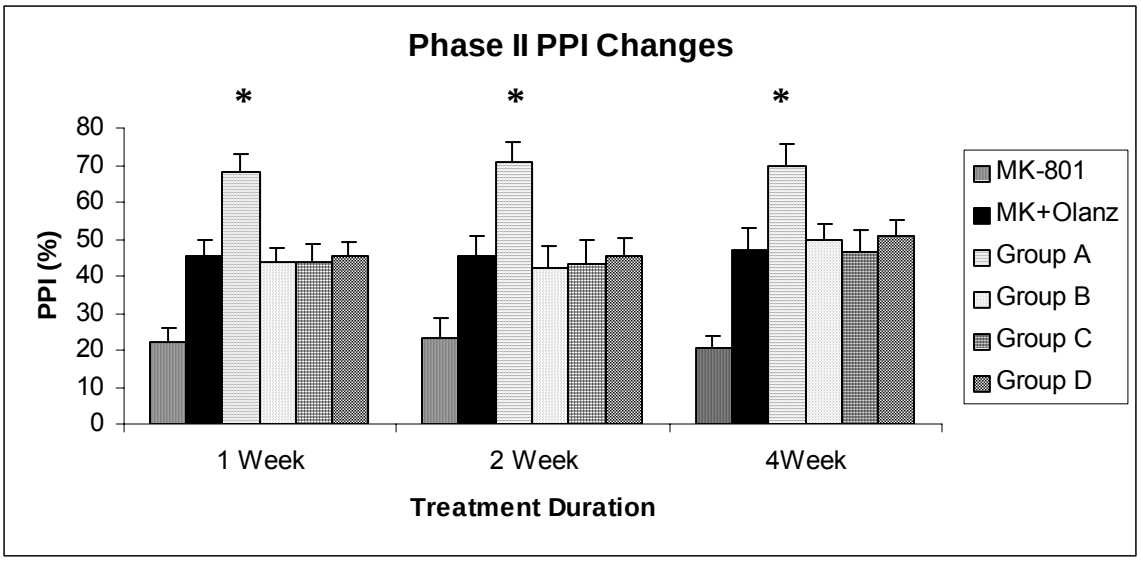


Fig. 39: Showing the effect of various drug treatments on the prepulse inhibition in rats. Each point signifies the PPI (mean ± SEM) for that group. For post hoc, Tukey's test was conducted (* $p < 0.001$ when compared to MK-801+ Olanzapine combination treatment group) [Note: The data for MK-801 and MK-801 + Olanzapine groups is from phase I experiment]

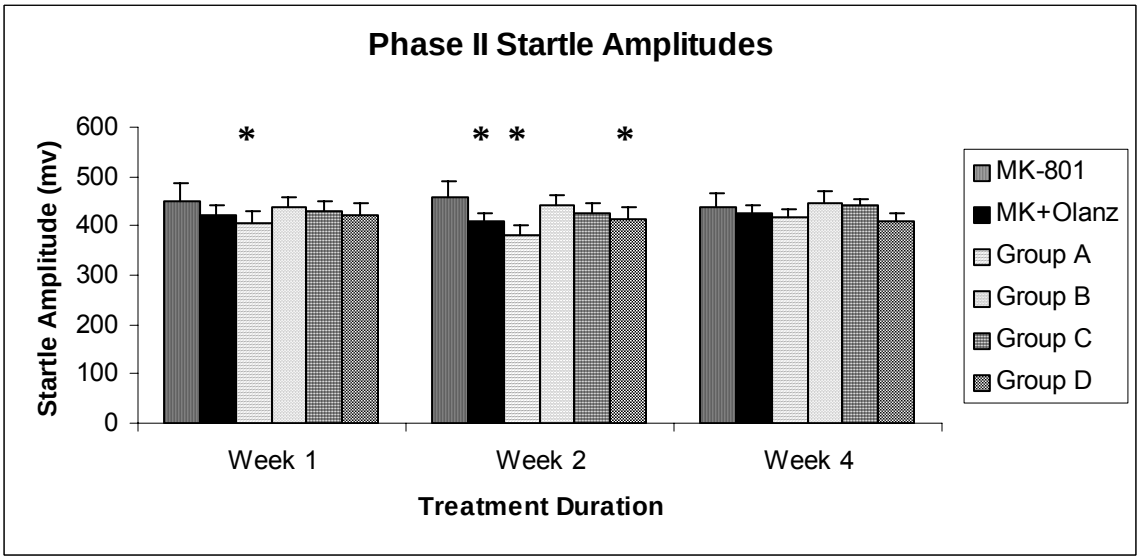


Fig. 40: Showing the effect of various drug treatments on the startle amplitude in rats. Each point signifies the Amplitude (mean ± SEM) for that group. For post hoc, Tukey's test was conducted (* signifies $p < 0.05$ compared to the MK-801 group for that duration of treatment). [Note: The data for MK-801 and MK-801 + Olanzapine groups is from phase I experiment]

Treatments: 1) MK-801 = 0.1 mg/kg on the day of the experiment; **2)** MK + Olan = Olanzapine 10 mg/kg/day for 4 weeks + MK-801 0.1 mg/kg on day of experiment; **3)** Group A = Prazosin 1.0 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment; **4)** Group B = Cirazoline 0.75 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment; **5)** Group C = Idazoxan 1.5 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment; **6)** Group D = Clonidine 0.2 mg/kg/day + Olanzapine 10 mg/kg/day, both for 4 weeks and MK-801 0.1 mg/kg on day of experiment.

DISCUSSION

In the first immunohistochemistry experiment I made an attempt to compare the effects of typical and atypical antipsychotics on IEG expression in the mPFC and LC areas of the brain. In the mPFC, four-weeks chronic treatment with the typical antipsychotic, haloperidol, reduced the number of nuclei immunoreactive for c-Fos, whereas chronic treatment with clozapine increased the number of nuclei immunoreactive for c-Fos. However, chronic treatment with risperidone, another atypical antipsychotic, had no effect on c-Fos immunoreactivity. Despite numerous publications on the acute effects of antipsychotics on IEG expression, there have been few previous studies of the effects of chronic treatment with antipsychotic drugs on IEG expression in the prefrontal cortex. In one study, Kontkanen et. al. (2002) investigate the effects of chronic treatment with clozapine and haloperidol for 17 days on *fos* and *jun* family genes by measuring mRNA levels 2 hours, 24 hours and 6 days after discontinuation of antipsychotic treatment. In their study, the mRNA expression after delays of 24 hours and 6 days after withdrawal of antipsychotic most likely reflect changes of IEG expression in response to the antipsychotic withdrawal. In my study, I perfused the animals 16 to 18 hours after the last injection. This was 6 to 8 hours before the time when the next dose would have been delivered, had treatment been continued. This time-point was selected to determine protein expression while plasma levels of antipsychotic were in the inter-dose trough in once daily dosing. Kontkanen et al. (2002) observations at 2 hours probably correspond most closely to mine as protein levels are expected to change less rapidly than mRNA levels. Consistent with our observation of downregulation of c-Fos immunoreactivity in the mPFC following chronic haloperidol treatment, they saw downregulation of *c-fos*

mRNA in the prefrontal cortex 2 hours after 17 days treatment with haloperidol (Kontkanen et al., 2002). However, while Kontkanen et. al. (2002) found significantly increased *c-fos* mRNA levels in the prefrontal cortex only 6 days after 17-days clozapine treatment, I found a significantly increase in the number of nuclei immunoreactive for c-Fos protein after 4-weeks chronic treatment with clozapine.

The difference in the duration of treatment may explain the difference in my results. Also, protein levels do not always correspond to mRNA levels as they are influenced by other factors such as rates of degradation. My observation of increased c-Fos protein immunoreactivity may reflect changes in protein processing that lead to increased protein levels in the absence of increased mRNA levels. These increases in Fos protein levels may have functional consequences for transcriptional regulation. While Kontakanen et. al. (2002) measured *fos* family gene mRNA levels in tissue samples, my measure was a count of immunoreactive nuclei detected above threshold level of intensity and is thus more sensitive to increases in numbers of cells expressing c-Fos immunoreactivity than to total levels of expression. Therefore it may be that the difference in our observations is due to a subpopulation of mPFC cells that express c-Fos immunoreactivity on chronic treatment with clozapine.

Although clozapine acutely activates Fos-like immunoreactivity in the LC (Dawe et. al., 2001; Ohashi et. al., 2000), chronic treatment with clozapine did not change c-Fos immunoreactivity in the LC. Neither did chronic treatment with haloperidol or risperidone. Haloperidol and clozapine are reported to acutely increase firing in the LC (Dinan and Aston-Jones, 1984; Nilsson et al., 2005; Ramirez and Wang, 1986; Souto et

al., 1979) , and chronic haloperidol, risperidone, and clozapine produce sustained increases in LC firing (Dinan and Aston-Jones, 1985; Nasif et al., 2000; Ramirez and Wang, 1986). As expression of c-Fos and Fos-like immediate early genes has been considered a marker of neuronal activation (Dragunow and Faull, 1989; Sagar et. al., 1988), it might have been expected that, as there are sustained increases in LC cell firing, there would be sustained increases in c-Fos immunoreactivity. However, in other brain regions, it has been observed that chronic treatment with antipsychotics can result in a return to control levels of expression of Fos-like immunoreactivity and this has been attributed to the development of tolerance (Sebens et. al., 1998). It may be that tolerance to the induction of c-Fos expression by neuronal activation develops in the LC.

In the mPFC, chronic haloperidol treatment upregulated expression of Egr-1 (also known as Krox-24 and zif268) immunoreactivity. However, the atypical antipsychotics did not significantly influence Egr-1 expression and none of the antipsychotics tested had any effect on Egr-2 expression. Once again chronic treatment with the typical antipsychotic had a qualitatively different effect from chronic treatment with the atypical antipsychotics. As it has been reported that Egr-1 expression in the mPFC is associated with consolidation of extinction of fear conditioning (Herry and Mons, 2004), the observation that chronic haloperidol treatment increases Egr-1 immunoreactivity in the mPFC leads to the testable prediction that chronic treatment with haloperidol, but not risperidone, will enhance consolidation of extinction of fear conditioning.

In the LC, all three antipsychotics investigated in the present study down regulated both Egr-1 and Egr-2 expression but increased TH expression. It had been suggested that *egr*

family immediate early genes are involved in regulation of TH expression, perhaps in close association with AP-1 complex transcription factors such as c-Fos (Papanikolaou and Sabban, 1999; Papanikolaou and Sabban, 2000; Nakashima et. al., 2003). However, the current finding of downregulation to both Egr-1 and Egr-2 despite increases in TH suggests that this is not the case for antipsychotic-induced expression of TH in the LC. This is consistent with a recent report on immobilization stress-induced TH expression which found that while Egr-1 was associated with TH expression in the adrenal medulla, in the LC there was no association between Egr-1 and TH expression (Sabban et al., 2004).

In the mPFC, only risperidone and clozapine, but not haloperidol produced increases in TH immunoreactivity. In the mPFC, the TH immunoreactive profiles counted are likely to correspond to axonal projections both from the LC, but also from the dopaminergic cells of the ventral tegmental area. The increase in the number of immunoreactive profiles detected on chronic treatment with the atypical antipsychotics may in part represent sprouting or branching of noradrenergic and dopaminergic fibres. It is also likely that the increase in the number of profiles counted reflects transport of newly synthesized TH protein from the cell bodies in the LC and ventral tegmental area resulting in more immunoreactive profiles being detected by our threshold sampling technique. In the LC, all three antipsychotics increased TH immunoreactivity. In LC the TH immunoreactive profiles counted most likely represent somatodendritic (bodies of TH producing cells) elements rather than the dendrites(cellular projections) alone. The increase in TH immunoreactivity was greater for the atypical antipsychotics risperidone (1 mg/kg/day) and clozapine (10 mg/kg/day) than for the typical antipsychotic

haloperidol (4 mg/kg/day). Between the atypical antipsychotics, clozapine (10 mg/kg/day) produced greater increases in TH immunoreactivity in the LC than risperidone (1 mg/kg/day). These data are consistent with the relative potencies of these antipsychotics in releasing noradrenaline in the prefrontal cortex (Westerink et al., 1998).

Although the effects of acute administration of antipsychotics on noradrenaline release have been extensively studied, to date few studies have investigated the effects of chronic antipsychotic treatment on noradrenaline release. One study on chronic risperidone administration found that, despite sustained increases in LC cell firing and contrary to the increase in TH immunoreactivity found in the present study, chronic treatment with risperidone reduced basal release of noradrenaline in the mPFC (Nasif et al., 2000). As beta-adrenoceptor-dependent mechanisms contribute to expression of Fos proteins in the cortex in response to various stimuli (Bing et al., 1992b; Bing et al., 1992a; Bing et al., 1991; Ohashi et al., 1998; Ohashi et al., 2000; Stone et al., 1991; Stone et al., 1995; Stone and Zhang, 1995), this reduction in noradrenaline release may contribute to an explanation for the absence of an effect of chronic risperidone treatment on c-Fos immunoreactivity in the mPFC. Alpha-2 adrenoceptor antagonism increases the firing rate of LC cells (Cedarbaum and Aghajanian, 1976; Freedman and Aghajanian, 1984). Clozapine has greater affinity for alpha-2 adrenoceptors and hence the greater effect of clozapine, as compared to risperidone, in increasing TH expression in the LC may relate to greater in vivo occupancy of alpha-2 adrenoceptors at the doses used in the present study (Schotte et. al., 1993).

The doses of antipsychotics were such that the atypical antipsychotic doses would be expected to generate peak plasma levels resulting in D2 occupancy levels corresponding approximately to that achieved by typical clinical doses (Kapur et al., 2005). In contrast, for haloperidol relative to clinical equivalence a high dose was administered. The literature had suggested that haloperidol, like the atypical antipsychotics, can dose-dependently induce LC firing and noradrenaline release in the PFC but that at doses of clinical equivalence haloperidol produces effects of smaller magnitude (Dinan and Aston-Jones, 1984; Nilsson et al., 2005; Westerink et al., 1998). Therefore, as the biochemical effects of low and high doses of haloperidol are qualitatively similar (Marcus et al., 2002), I chose to administer a relatively high dose of haloperidol in order to test whether the effects of chronic haloperidol on the locus coeruleus-prefrontal cortical system are qualitatively different, rather than merely quantitatively different, from those of atypical antipsychotics. Despite this haloperidol had less effect on TH immunoreactivity in the LC, failed to influence TH immunoreactivity in the mPFC, and had effects opposite to those of clozapine on c-Fos immunoreactivity in the mPFC.

In the other immunohistochemistry experiment I tried to look at the effect of different dosages and different treatment durations of antipsychotic drug olanzapine on IEG expression. In the LC and mPFC, acute (4 hrs) treatment with olanzapine increased the number of nuclei immunoreactive for c-Fos protein. This is consistent with previous reports (Dawe et al., 2001; Ohashi et al., 2000; Robertson and Fibiger, 1996). Acute administration of olanzapine activates the LC (Dawe et al., 2001; Seager et al., 2004). As neuronal activity is associated with c-Fos expression (Dragunow and Faull, 1989; Morgan and Curran, 1991; Sagar et al., 1988), activation of LC cells may be sufficient to

induce c-Fos expression. Acute administration of olanzapine also induces noradrenaline release in the prefrontal cortex (Li et al., 1998; Westerink et al., 1998). LC activation can lead to β-adrenoceptor-dependent increases in Fos-like immunoreactivity in the cortex (Bing et al., 1992; Bing et al., 1991; Stone et al., 1995; Stone and Zhang, 1995). Also the induction of Fos-like immunoreactivity in the mPFC by acute administration of olanzapine was seen to be blocked by a β-adrenoceptor antagonist (Ohashi et al., 2000). Hence it is likely that the increase in c-Fos expression seen in the mPFC is, at least in part, a consequence of increased noradrenaline release and perhaps the action of noradrenaline at β-adrenoceptors. Alternatively, olanzapine may lead to activation of the mPFC, which in turn leads to activation of the LC. However, this is unlikely as self-stimulation of the mPFC did not lead to c-Fos expression in the LC (Arvanitogiannis et al., 2000).

On treatment with olanzapine for 1 week and 2 weeks, increases in the numbers of c-Fos immunoreactive nuclei were sustained in both the LC and mPFC. However, with 4-weeks treatment the numbers of c-Fos immunoreactive nuclei in the mPFC of olanzapine-treated animals were not significantly different from those in saline-treated controls. Likewise in the LC, at all but the highest dose of olanzapine, there was no difference from saline-treated controls with 4-weeks treatment. Treatment with 15 mg/kg/day olanzapine for 4 weeks reduced c-Fos immunoreactivity in the LC. Sebens et al (1998) had found a down-regulation of c-Fos expression in the prefrontal cortex of rats treated daily for 3 weeks with 5 mg/kg olanzapine and then challenged with 5 mg/kg olanzapine as a control in study of haloperidol and clozapine cross-tolerance. In contrast i saw no evidence of significant down regulation of c-Fos in the mPFC. The difference may be due the dosing regimen. Sebens et. al (1998) administered once daily intraperitoneal injections and

perfused the animals 2 hours after a challenge injection, whereas i administered the olanzpaine by continuous infusion by subcutaneous osmotic minipump. Sebens et. al. (1998) attributed the down-regulation of c-Fos expression to the development of tolerance. Development of tolerance may also explain the reduction of c-Fos expression to control levels observed in the present study.

In both the mPFC and LC, at most of the time-points studied from 4 hours to 4 weeks, the patterns of change in c-Fos and c-Jun immunoreactivity were similar. This is to be expected as c-Fos and c-Jun are both intimately involved in forming the AP-1 complex and is consistent with previous reports that their expression in response to various stimuli is congruent (Herdegen and Leah, 1998). At notable exception in the present study, is the pattern of expression in the LC on 4 weeks treatment with olanzapine. At this time-point, the increase in c-Jun expression is maintained but the expression of c-Fos returns to control levels or less at the highest dose. This may be evidence that, at least in the LC, different signaling pathways regulate the expression of c-Fos and c-Jun.

ATF-2 is constitutively expressed in many cells throughout the mammalian nervous system (Herdegen and Leah, 1998) but nevertheless treatment with olanzapine still increased the number of ATF-2-positive nuclei detected. Our threshold detection method of counting immunoreactive nuclei may not be detecting de novo expression of ATF-2 but rather activated nuclei expressing higher levels of ATF-2. In the LC, olanzapine treatment rapidly and persistently unregulated the number of ATF-2 immunoreactive nuclei. The ATF-2 expression was up-regulated at all treatment durations. Intermediate doses of olanzapine (4 mg/kg/day and 8 mg/kg/day) were most effective at inducing both

ATF-2 and TH expression. Administration of doses in this range by osmotic minipump in rats is likely to mimic plasma concentrations of olanzapine seen in humans (Seager et al., 2005; Seager et al., 2004). It may be that the expression of ATF-2 leads to induction of increased TH expression as Suzuki et al. (2000) showed that ATF-2 is a transcription factor involved in the regulation of expression of the TH gene.

In the mPFC, the intermediate doses of olanzapine increased ATF-2 immunoreactivity across all treatment durations, although less robustly than in the LC. However, the lowest dose (2 mg/kg/day) initially suppressed ATF-2 expression after treatment for 4 hrs and 1 week but later increased ATF-2 expression. The highest dose of olanzapine (15 mg/kg/day) was less effective than intermediate doses in inducing ATF-2 expression, especially on longer-term treatment. This tendency towards an inverse U-shaped dose-dependence suggests that multiple mechanisms triggered by the complex pharmacology of olanzapine may be contributing to regulation of ATF-2 expression.

ATF-2 may play a role in the induction of c-Jun. In some systems, it induces c-Jun in an AP-1 independent manner (van Dam et al., 1995). Also c-jun and ATF-2 can be acted upon by the same c-Jun N-terminal kinases, suggesting involvement of the same secondary messenger pathways in regulation of their expression (Derijard et al., 1994). The increase in the expression of ATF-2 in the LC is much more robust than in the mPFC. It may be that the increased ATF-2 expression in the LC at 4 weeks helps in the direct induction of c-Jun. This may contribute to the sustained expression of c-Jun, while the expression of the other AP-1 complex IEG, c-Fos, drops to control levels.

There is an overall downregulation of both Egr-1 (also known as Krox-24) and Egr-2 (also known as Krox-20) in both the mPFC and LC across all treatment durations. In the present study, the Egr-1 expression was inversely proportional to the dose of olanzapine, while the expression of Egr-2 was directly proportional to the dose of olanzapine. The explanation for this is at present unclear. However, it is noted that Egr-1 can auto-regulate its transcription by binding with high affinity to the response element in its own promoter, while Egr-2 transcription is suppressed by c-Fos in some systems (Gius et. al., 1990). This may contribute to the observed pattern of expression.

Olanzapine increased the number of TH immunoreactive profiles counted in both the LC and mPFC. In the LC, the TH immunoreactive profiles counted most probably represent mainly somatodendritic and axonal elements of LC cells. In the mPFC, the TH immunoreactive profiles counted likely represent mostly axonal projections from the LC and the dopaminergic cells of the ventral tegmental area. In the LC, the increase in the number of TH immunoreactive profiles appeared with only 4 hrs of olanzapine treatment suggesting that the increase is unlikely to be due to growth of new dendrites or axons by TH expressing cells. It is possible that this represents induction of *de novo* expression of TH , especially as tyrosine hydroxylase mRNA may be present in dendrites (Dumas et al., 1990) allowing for rapid somatodendritic translation of protein. Alternatively, this may reflect redistribution of TH to fine dendritic or axonal profiles that would have otherwise fallen below our detection threshold.

In contrast, Ordway and Szebeni (2004) found only modest effects of olanzapine treatment alone on TH immunoreactive protein in the LC measured by quantitative

Western blotting. The increase in TH was seen with 18 days of olanzapine treatment at 5 mg/kg bid but not with treatment for 12 days or with 3 mg/kg/day for 18 days (Ordway and Szebeni, 2004). It is possible that the changes observed in the numbers of TH immunoreactive profiles that we observed reflect redistribution of TH rather than an increase in the synthesis of TH. As this redistribution does not necessarily correlate with increased overall expression of TH, our data are not inconsistent with those of Ordway and Szebeni (2004). Alternatively, continuous infusion of olanzapine by osmotic minipump in the present study may have resulted in earlier increases in TH expression. Other stimuli that trigger LC cell activation, for example stress, have been reported to result in rapid (less than 24 hrs) induction of TH expression in LC cells (Melia and Duman, 1991; Serova et al., 1999; Smith et al., 1991b; Zigmond et al., 1974). As olanzapine activates the LC (Dawe et al., 2001), it might be expected to rapidly induce TH expression in the LC, especially if activation is sustained by continuous infusion. As there is release of noradrenaline within the LC consistent with somatodendritic release (Pudovkina et al., 2001; van Gaalen et al., 1997), early increases in somatodendritic expression of TH may influence LC activity through increased local release of noradrenaline within the LC.

In the mPFC, increases in the number of TH immunoreactive profiles relative to the saline treated control group were evident only after 2 and 4-weeks administration of olanzapine. This is consistent with studies of TH expression induced in the LC by other stimuli, such as nicotine administration, which suggest that it takes several weeks for newly synthesized TH to be transported along the projections of the LC to the forebrain (Mitchell et al., 1993). The TH immunoreactive profiles detected in the mPFC in the

present study could also arise from the dopaminergic innervation of the mPFC by the ventral tegmental area, however levels of noradrenaline in the mPFC exceed those of dopamine (Fadda et al., 1984) so it is likely that many of the TH immunoreactive profiles detected represent noradrenergic fibres arising from the LC. Together with the finding that chronic olanzapine treatment for 3 weeks produced sustained increases in LC firing and bursting (Seager et al., 2005), this may suggest a mechanism to support further sustained increases in noradrenaline release in the mPFC on chronic treatment with olanzapine. However, while TH immunoreactivity in the mPFC continued to increase even with 4 weeks treatment, c-Fos expression dropped again after 4 weeks of administration of olanzapine. This suggests that on prolonged treatment tolerance or other compensatory mechanisms are activated that suppress the noradrenaline-induced activation of c-Fos in the mPFC. Perhaps these mechanisms include suppression of the noradrenaline release despite sustained increases in LC firing. To date microdialysis studies have focused on the effects of acute treatment with olanzapine on noradrenaline release in the mPFC. The present data suggest the need for further microdialysis studies to investigate the release of noradrenaline on chronic treatment with olanzapine.

In the experiments on LI in *chakragati* (*ckr*) mice we see that i) the pre-exposed homozygous mice showed significantly lower suppression ratio than the pre-exposed wild type and pre-exposed heterozygous mice and ii) the difference in the suppression between the pre-exposed and non pre-exposed is reduced significantly in homozygous mice as compared to the wild type and the heterozygous mice. This second observation is brought about mainly by a reduction in the suppression ratio of the pre-exposed mice. The non pre-exposed ones show a similar suppression ratio to the ones shown by the non

pre-exposed wild type and heterozygous mice. This indicates a different conditioned suppression for pre-exposed and non pre-exposed animals. Over all the homozygous *ckr* mice shows significant differences in the results of LI as compared to the heterozygous and the wild type groups. The results are similar to those seen in schizophrenics or other animal models, which have been treated with amphetamine or MK-801.

Different experiments have shown different results with respect to LI disruption in animals. In an experiment by Killcross et. al. (1994), it is proposed that disruption of LI is accompanied by nondifferential conditioned suppression in the PE and NPE groups. In this experiment amphetamine was used to produce the LI. In other experiments, like the one conducted by Weiner et. al., (1997) it is proposed that there is differential conditioned suppression in PE and NPE groups with the PE group showing better learning. In this experiment also the scientists employed amphetamine to produce LI. My results with *ckr* mice are similar to the ones shown by Weiner et al (1997), and I could possibly use the same explanation of "Switching Model" of LI to explain my results. According to this model interventions resulting in LI changes shift the relative balance between the behavioral impact of pre-exposure and conditioning. This effect is restricted only to PE groups and not to the NPE ones. Manipulating the pre-exposure repetitions and their duration on one hand and the intensity of conditioning (in my case the shock intensity) can swing the balance of suppression in either direction. For example increasing the shock intensity may lead increase in the conditioning impact. Weiner et al (1997) also go on to suggest that a thorough parametric study should be conducted so as to assess the impact of all these variables in any model of LI. I realize the need to do this

and I do plan to conduct such an experiment in future to substantiate the "switching model" of LI in the *ckr* mice.

In the experiments with the *ckr* mice I also observed that the homozygous mice showed significant reduction in the PPI compared to the wild type and the heterozygous mice. This result is similar to the other PPI results, which are seen in other genetically manipulated mouse models of schizophrenia like V lbR knock out mice (Egashira et. al., 2005), or the corticotropin- releasing factor (CRF) over-expressing mice (Dirks et. al., 2003) or the mGluR5 knockout mice (Brody et. al., 2004). The homozygous animals seem to have sensory-motor gating problems and there is a definite attenuation of PPI in these animals. The results hold true over different prepulse intensities. It is interesting to note that the genetic mutation does not seem to alter the baseline startle amplitude significantly in the homozygous mice. It seems unlikely that the decrease in PPI is merely an artifact of an alteration in the baseline startle reactivity. Rather it appears that the *ckr* mice have a veritable disruption of sensorimotor gating.

As discussed by Torres et al (2004), the *ckr* mice seem to have certain abnormalities in brain anatomy. To test the fact that these mice did not have any hearing defect, I conducted the second test where I altered the time gap between the prepulse and the pulse stimuli. The results show that these mice do not have any hearing defect. The gap of 25 ms is too small for the brain circuits to recognize the prepulse and act on it. Before this happens the pulse tone is received and as such there is almost negligible prepulse inhibition. If the mice had any hearing defect they would not respond to either the prepulse or the pulse tone. The results with the 100 ms gap and 175 ms gap are almost

identical and show prepulse inhibition. It demonstrates the fact that the animals are able to hear the tones properly and process the stimuli in a systematic pattern, thus showing the prepulse inhibition.

Detailed examination of the *ckr* mice brain anatomy (Torres et al, 2004) showed that these animals have increased size of lateral ventricles. This increase was in the tune of approximately 1500 %. This is accompanied along with a loss of individual myelinated axons bordering these ventricles and agenesis of the corpus callosum. Apart from these changes no other gross abnormality was noticed in the brain structure. It is highly unlikely that the circling behaviour of the mice and these results in the PPI and LI experiments are related to the ventricular enlargement, because the enlargement is noticed in heterozygous mice also but they don't show similar phenotypic character or this behavior in PPI test.

Ventricle enlargement has been noticed in patients of schizophrenia also (Harrison, 1999). Torres et. al., (2004) have gone to suggest that even in humans there are relatives of schizophrenics who are not affected by schizophrenic symptoms but they do have ventricular enlargement like the schizophrenics. This seems to suggest that a similar pattern is observed in the *ckr* mice where the heterozygous and homozygous mice show similar anatomical changes but different schizophrenic characters.

Ckr mice show hyperactivity similar to the animals treated with MK-801 or PCP. Even in humans, schizophrenia is sometimes associated with hyperactivity, which usually constitutes the positive symptoms of the disease. I have tried to test the validity of *ckr*

mouse as a model for schizophrenic deficits in PPI and LI. PPI and LI, and have got positive results. The animal looks very promising as a model of schizophrenia. It has shown some close parallels with human schizophrenics as well as other animal models of schizophrenia. To take my experiments further I tested the effect of anti psychotic drugs on the PPI of *ckr* mice. It was found that the drugs did not seem to have any significant effect on the PPI in the wild type mice. In the homozygous strain, although all the drugs showed improvement in the PPI, significant results were obtained only with clozapine. All three doses of clozapine improved the PPI significantly. High dose risperidone also showed promising result but fell a little short of significant levels. These results seem to corroborate past studies in animal models with disrupted PPI, where these drugs improve the PPI deficits, clozapine improving phencyclidine–induced deficits in PPI (Bakshi et al, 1994); haloperidol improving MK-801 induced PPI deficits (Feifel and Priebe, 1999); risperidone, and clozapine improving neonatal ventral hippocampal lesion induced PPI deficits (Le Pen and Moreau, 2002). I did not see any significant effect of these drugs on the PPI in wild-type mice. The results are similar to the study done by Egashira et al. (2005) and Brody et al. (2004). However a few studies are not consistant with our results. In wild type C57BL/6J mice haloperidol is seen to facilitate PPI (McCaughran et al, 1997). Risperidone appears to produce dose –dependent improvement in PPI (Ouagazzal et al, 2001), although this particular finding is not unequivocal (Oliver et al, 2001). Dirks et al (2003) found that high doses of clozapine, 10 mg/kg/day , reduced PPI in wild-type mice. The author has explained this effect by stating that there were concerns about the greatly reduced startle reactivity caused in those particular mice by clozapine. The author also talks about the difference in the basal PPI levels between his study and other studies, which may account for the unusual PPI impairing effect of clozapine observed in their

experiment. Other differences between the results for the wild type mice in the experiments and other experiments can be explained by factors as mentioned by Dirks et al. (2003). The differences may be due to substrain differences in drug responses, and / or differences in stimulus parameters, PPI parameters and startle measurement methods.

Amongst all the antipsychotics only the high dose clozapine (10 mg/kg/day) had any significant effect on the startle amplitudes of the mice. This treatment significantly reduced the startle amplitude in both the wild-type as well as the homozygous strain. The small (1 mg/kg/day) and medium (4 mg/kg/day) dosages don't show any effect on the startle amplitude. A few studies have reported similar results as me on this account. Egashira et al. (2005) showed that haloperidol and risperidone had no significant effect on wild-type as well as genetically modified mice, while high dosage of clozapine reduced the startle, but only in the knockout mice. Similar results were found with clozapine treatment in the study done by Brody et al. (2004) on mGluR5 knockout mice and Bakshi et al. (1994) on MK-801 treated rats. But Le Pen and Moreau, (2002) showed opposite results with all the three drugs clozapine, risperidone and haloperidol showing dose-dependant reduction in the startle in sham-operated and brain lesioned animals. Though in their case the animals used were rats. Studies on genetically modified mice have generally found similar results to mine (Egashira et al, 2005; Brody et al, 2004) and as such we can suggest that species difference can affect the influence of these drugs on the startle amplitude.

Still this is an interesting data because this helps me in dissociating the PPI enhancing effect of clozapine from the effects on startle amplitude. Firstly, clozapine (10 mg/kg/day)

reduces amplitude in both wild-type and homozygous strain while the PPI is significantly raised only in homozygous mice. Secondly the low and medium dosages of clozapine, even though they don't affect the startle amplitude, they still significantly enhance PPI in homozygous mice. Clozapine seems to affect the sensori-motor gating the homozygous mice and its effect on the PPI doesn't seem to be due to the floor effect on the startle amplitude. Even in clinical settings clozapine is seen to cause a better improvement in the PPI deficits of schizophrenic patients, when compared to other atypical antipsychotics and typical antipsychotics (Oranje et. al, 2002).

I proceeded to test the involvement of the adrenergic system in the PPI deficit of the homogenous *ckr* mice. I used alpha-1 adrenergic drugs cirazoline and prazosin for this purpose. I saw that on their own, these drugs did not have any significant effect on PPI, both in wild-type as well as the homozygous *ckr* strain. But when the α-1 antagonist drug prazosin was combined with clozapine it managed to give higher PPIs than clozapine alone. An additive effect was observed in this case. Even when given alone, prazosin increased the baseline PPI of the homozygous mice, but just fell short of significant levels. My results are similar in some respect to those carried out by Bakshi and Geyer (1999). In their experiment it was seen that although prazosin did not improved PPI in control rats, it did increase the PPI in animals treated with MK-801. Another study by Bakshi and Geyer (1997) mentions the ability of prazosin to significantly raise PPI in PCP treated rats. Carasso et al. (1998) showed that prazosin reversed disruptions in PPI produced by alpha1 agonist cirazoline. But one study (Powell et al., 2005) mentions the contrary result that prazosin was not able to increase PPI in yohimbine treated rats. The author goes on to explain that they got this result because in their opinion, yohimbine

disrupted PPI via 5-HT_{1A} receptors and not adrenergic receptors. Different PPI models can be created using the involvement of various neuroreceptors. In the case of *ckr* mice it is suggested that the alpha1 adrenergic receptors are involved. My experiments show that although cirazoline reduced PPI, the results were not significant. Previously Shilling et al (2004) and Carasso et al. (1998) had found that cirazoline decreases PPI significantly. But these studies were again in rats. *Ckr* mice have an inherently higher locomotor activity. This could have something to do with the brain levels of noradrenaline. These could in turn affect the alpha-1 adrenoceptors so as to diminish the effect of this dose of cirazoline.

For the startle amplitude it was seen that for both wild-type and homozygous strains, high dose clozapine, alone and in combination with prazosin, diminishes the startle amplitude. These results are consistent with my discussion of the effects of clozapine on the startle amplitude, as above. Addition of prazosin decreases the startle amplitude even further than clozapine alone. Prazosin alone seems to cause a small trend towards decrease in the startle amplitude, but it is not significant. This confirms earlier findings by Bakshi and Geyer (1997, 1999). Another study mentions no significant effect of prazosin on the startle amplitude (Powell et al., 2005), although here it also did partially decrease the startle amplitude. As found in previous study Shilling et. al., 2004) cirazoline did not have any effect on the startle amplitude.

In the rat experiments I investigated the role of antipsychotics and adrenergic drugs on spatial memory (tested by Morris water maze) and on prepulse inhibition in MK-801 treated rats. I tested the effect of all the drug treatments on the spatial memory in rats

treated with MK-801. We saw that MK-801 administration greatly disrupted the performance of the animals in comparison to the controls. The latency to find the platform was significantly increased and the swim distance was also increased . My results are simlar to the results obtained in previous studies (Pitkanen et. al., 1995; Ahlander et. al., 1999; Winshaw and Auer, 1989; Lukoyanov and Paula-Barbosa, 2000; Heale and Harley, 1990). The combination of haloperidol to MK-801 further disrupt the performance of the animals. This is to be expected as haloperidol administered alone is also known to increase latency in finding the platform in rats (Terry et. al., 2002; 2003; Wilson et. al., 2003). These rats showed the slowest swim speed amongst all the groups which suggests that this may be due to sedation or motor impairment. Olanzapine was able to reverse the effects of MK-801 and improve the performance of the rats. In experiments done by Terry et al (2003), there was no improvement in the performance of animals after 45 days of treatment. These experiments were conducted after 4 days of wash-out after the last dosage. In my case, I performed the experiments without waiting for any wash out phase. Also Terry et al (2003) saw that by the last trial on the last day, the performance of the rats had improved a lot. It is possible that olanzapine on its own, does not effect the performance, but is able to improve MK-801 induced deficits.

The MK-801 induced disruptions in the performance of rats was reversed to a greater extent in rats who had been pretreated with the adrenergic drugs alpha-2 antagonist, idazoxan, or the alpha-1 agonist, cirazoline, along with olanzapine, rather than just olanzapine administration alone. The performance of the animals deteriorated when olanzapine was combined either with alpha-2 agonist clonidine, or the alpha-1 antagonist, prazosin. Although out of all these effects only the improvement by idazoxan was

significant. This is not surprising since on their own alpha-2 antagonists like dexefaroxan are known to improve spatial memory processes (Chopin et. al., 2002). In patient studies (Frith et. al., 1985; Riekkinen et. al., 1999), clonidine was shown to be detrimental to memory performances. In my study, the alpha-2 drugs show similar effects even when they are combined with olanzapine, with idazoxan improving performance and clonidine depreciating the performance of the animals. Similarly the actions of alpha-1 drugs are consistant with previous studies, that showed cirazoline improving performance (another alpha1 agonist ST587 showing similar results, Riekkinen et. al., 1997). The alpha-2 antagonist, idazoxan, showed an additive effect in the improvement of spatial memory in water maze.

For PPI I started by treating the rats with MK-801 and the antipsychotic drugs haloperidol and olanzapine. MK-801 significantly disrupted PPI in rats, confirming previous results obtained in various experiments (Mansbach and Geyer, 1989; Johansson et al. 1995). It was found that haloperidol treatment could not reverse this effect of MK-801 even after 4 weeks of treatment. This is in conformity with previous studies (Geyer et. al. 1990; Keith et. al. 1991; Feifel and Priebe, 1999). The results with olanzapine were different and this drug seemed to significantly improve the PPI deficit caused by MK-801. My results are similar to the ones obtained by Bakshi and Geyer (1995) in this regards.

MK-801 in general, significantly increased the startle amplitude in animals. This is in accordance to previous studies (Feifel and Priebe, 1999; Schultz et. al. 2001). The results obtained for haloperidol, i.e., no significant effect on MK-801 induced increase in the startle amplitude, and for olanzapine, i.e., decrease in MK-801 induced increase in startle

amplitude also agree with previous studies (Bakshi and Geyer, 1995; Feifel and Priebe, 1999). Olanzapine is also known to diminish startle activity in PCP treated animals. It matches the profile of clozapine in this matter. Combined with it's effect on MK-801 induced changes, over all olanzapine can be said to disrupt PPI effects of NMDA receptor antagonists in general.

Following confirmation of previous findings I further investigated the effects of adrenergic drugs on the PPI and their interaction with olanzapine. It was noticed that only the combination of olanzapine and prazosin was able to significantly raise the PPI levels when compared to just olanzapine treatment in rats who's PPI had been disrupted by MK-801. This change was seen throughout the four week treatment period. Combining olanzapine with other drugs like cirazoline, clonidine and idazoxan did not alter the MK-801 induced PPI disruption significantly when compared to administration of olanzapine alone. However these groups still showed marked improvement over treatment with MK-801 alone. Cirazoline has been to known to reduce PPI in animals on its own (Shilling et. al., 2004). It was seen that the presence of olanzapine prevents cirazoline from decreasing the PPI in conjunction with MK-801. Even alpha-2 receptors knockout, has been shown to disrupt PPI in studies by Lahdesmaki et. al. (2004) and Sallinen et. al. (1998). Sallinen et. al. (1998) went on to also show that alpha-2 receptor over-expression, which can be considered a form of agonism, led to opposite effects, i.e., increase in PPI and decrease in the startle reactivity. In this study, the alpha-2 antagonist, Idazoxan, failed to significantly reduce the PPI and alpha-2 agonist clonidine, failed to increase PPI, in the presence of olanzapine. Considering all these data together, the additive effect of

adrenergic drugs with the antipsychotic drug olanzapine was seen only in the case on prazosin.

For startle amplitudes at 1 week treatment duration, the combination of olanzapine + prazosin + Mk-801 had a significantly lower startle amplitude compared to MK-801 alone treatment. At 2 week treatment duration, olanzapine + MK-801, olanzapine + prazosin + MK-801 combination and olanzapine + clonidine + MK-801 combinations showed significant decrease in startle amplitudes as compared to that of MK-801 treated animals. Acting alone, prazosin is not known to decrease the startle amplitude, but in PPI disruptions caused by yohimbine (Powell et. al., 2005) or PCP (Bakshi and Geyer, 1997), prazosin was seen to decrease the high startle amplitude. In my experiment, it was seen that for the MK-801 induced increase in startle amplitude prazosin plays a complimentary role with olanzapine in lowering startle amplitude.

CONCLUSIONS

The present study on change in IEG expression demonstrated that, despite qualitatively similar effects of acute treatment with atypical and typical antipsychotics on LC cell firing and Fos-like immunoreactivity in the prefrontal cortex, atypical and typical antipsychotics differ qualitatively in their chronic effects on immediate early gene and TH expression in the mPFC and LC. In particular, it was observed that chronic treatment with the atypical antipsychotics, risperidone and clozapine, produce greater increases in TH expression in the LC and mPFC than the typical antipsychotic, haloperidol. Further

studies are required to understand how these increase in TH immunoreactivity related to noradrenaline release in the mPFC after chronic treatment with antipsychotics. Activity-dependent increases in noradrenaline release in the mPFC may contribute to the greater efficacy of atypical antipsychotics against the negative and cognitive symptoms of schizophrenia. Although for many years it has been generally assumed that antipsychotics have a delayed onset of action, recent studied have questioned this delayed onset hypothesis and suggest that antipsychotics can have clinical effects as early as 2 hrs after initiation of treatment but that magnitude of the actions can increase over time (Agid et. al., 2003; Kapur et. al., 2005). In my study I have charted some of the effects of different olanzapine doses and treatment durations on IEG and tyrosine hydroxylase protein expression in the mPFC and LC of the rat. While there are immediate effects of olanzapine treatment, there are also delayed dose-dependent adaptations in the patterns of expression, especially between 2 and 4 weeks of treatment in the mPFC. Future investigation of how changes in IEG and tyrosine hydroxylase expression relate in the mPFC relate to prefrontal cortical dependent behaviours may further understanding of how expression of these proteins relates to the mechanisms of action of olanzapine and other atypical antipsychotic drugs

I showed that *ckr* mice have disrupted latent inhibition and prepulse inhibition. These effects were most likely not due to anatomical abnormality but were attributable to sensorimotor gating defects. It was further shown that typical and atypical antipsychotics effect PPI in the *ckr* mice differently. Atypical antipsychotics were more successful in reversing the PPI defects than the typical ones. Over all the *ckr* mice has given indication that in future it could serve as a useful animal model of schizophrenia.

The experiments with adrenergic drugs, both in *ckr* mice as well as the MK-801 induced deficit model in rats show an additive effect of alpha-1 antagonist, prazosin, and atypical antipsychotics in reversing PPI deficits. In the case of the spatial memory tests in rats, there seemed to be additive effects of the alpha-2 antagonist, idazoxan, with the atypical antipsychotic drug, in improving the water maze performance.

Starting from IEG expression to behavior testing in animals, a role for adrenergic system is visible in the pathopsysiology as well as treatment of schizophrenia. The additive effects of adrenergic drugs to the atypical antipsychotic drugs is encouraging and with further studies has the potential to develop into a novel therapeutic regime.

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1. Chronic high-dose haloperidol has qualitatively similar effects to risperidone and clozapine on immediate-early gene and tyrosine hydroxylase expression in the rat locus coeruleus but not medial prefrontal cortex.
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Neurosci Res. 2007 Jan;57(1):17-28. Epub 2006 Oct 5.

2. Effects of short term and chronic olanzapine treatment on immediate early gene protein and tyrosine hydroxylase immunorectivity in the ra locus coeruleus and medial prefrontal cortex.
Vivek Verma, Kurt Rasmussen and G.S Dawe
Neuroscience. 2006 Dec 1;143(2):573-85. Epub 2006 Sep 18.

3. Propranolol blocks chronic risperidone treatment-induced enhancement of spatial working memory performance of rats in a delayed matching-to-place water maze task.
Lim EP, **Verma V**, Nagarajah R, Dawe GS
Psychopharmacology (Berl). 2007 Jan 16; [Epub ahead of print]