ROLE OF SYNAPSIN II IN NEURODEVELOPMENT

ROLE OF SYNAPSIN II IN NEURODEVELOPMENT: DELINEATING THE ROLE OF DEVELOPMENTAL MEDIAL PREFRONTAL CORTICAL SYNAPSIN II REDUCTIONS IN THE PATHOPHYSIOLOGY OF SCHIZOPHRENIA

By

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TITLE: Role of Synapsin II in Neurodevelopment: Delineating the Role of Developmental Medial Prefrontal Cortical Synapsin II Reductions in the Pathophysiology of Schizophrenia

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ABSTRACT

Synapsins are primarily neuron-specific proteins critical for neurotransmission, synaptogenesis and synapse maintenance. Synapsin II has been specifically linked with increased susceptibility towards developing schizophrenia. Reduced synapsin II mRNA levels were found in the dorsolateral prefrontal cortex (PFC) of patients with schizophrenia. Moreover, synapsin II knockdown in the medial PFC (mPFC) of the adult rat was previously shown to cause schizophrenia-like behaviour and altered expression levels of vesicular proteins involved in glutamatergic and GABAergic signaling within the mPFC.

The study of schizophrenia in recent years has shifted to focus on neurodevelopmental players which influence disease outcome. This study was designed to establish the link between neurodevelopmental dysregulation of synapsin II and schizophrenia. Specific knockdown of synapsin II was performed in the mPFC at postnatal day (PD) 7 and PD 17-23. Schizophrenia-like behavioural abnormalities were assessed at pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages. Protein estimation of vesicular transporters involved in glutamate, GABA, and dopamine neurotransmitter systems were also assessed in the mPFC.

Results from this study indicate (1) synapsin II knockdown during PD 17-23, but not PD 7, caused lasting schizophrenia-like abnormalities (2) abnormalities exhibited permanence at pre-pubertal and post-pubertal stages, and manifested as a function of brain development, (3) behavioural abnormalities were reminiscent of symptoms in established animal models of schizophrenia (i.e. deficits in prepulse inhibition, social

withdrawal, locomotor hyperactivity), (4) neurodevelopmental synapsin II alterations induced hypoactive glutamatergic activity through decreased synapsin IIa expression levels (pre-pubertal) and decreased VGLUT-2 expression levels (post-pubertal), and (5) acute olanzapine treatment effectively attenuated schizophrenia-like abnormalities through normalized synapsin IIa expression levels (pre-pubertal) and increased $GAD_{65/67}$ expression levels (post-pubertal).

Results show the causal link between synapsin II expression during critical neurodevelopmental stages and schizophrenia. Additionally, evidence has been provided for the face, construct, and predictive validities of this newly developed animal model of schizophrenia.

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A quote from Flavia Weedn:

Some people come into our lives and quickly go. Some stay for awhile, leave footprints on our hearts, and we are never, ever the same.

Thank you for leaving your footprint on my life.

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5-HT	5-serotonin-2
aCSF	artificial cerebrospinal fluid
ANOVA	analysis of variance
A/P	anterior-posterior
AP-2α	activator protein-2α
AS	antisense deoxyoligonucleotides
ATP	adenosine triphosphate
BDNF	brain-derived neurotrophic factor
CaMK	Ca^{2+}/calmodulin-dependent protein kinase
cAMP	cyclic adenosine 3', 5'-monophosphate
CATIE	clinical antipsychotic trials of intervention effectiveness
Cdk	cyclin-dependent kinase
cDNA	complementary DNA
CNS	central nervous system
CpG	C-phosphate-G
DAT	dopamine transporter
DISC-1	disrupted-in-schizophrenia 1
DNA	deoxyribonucleic acid
DTNBP-1	dystobrevin-binding protein 1/dysbindin
D/V	dorsal-ventral
E12	embryonic day 12

EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
EGR	early growth response factor
EPS	extrapyramidal side effects
EUFEST	european first-episode schizophrenia trial
GABA	gamma-Aminobutyric acid
GAD	glutamic acid decarboxylase
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GPe	globus pallidus extermal
GPi	globus pallidus internal
HPLC	high-performance liquid chromatography
M/L	medial-lateral
PEA	polyoma enhancer activator
PCP	phencyclidine
PKA	protein kinase A
LSD	lysergic acid diethylamide
LTP	long-term potentiation
MAP	mitogen-activated protein
MC	mesocortical
MIA	maternal immune activation
ML	mesolimbic
MK-801	dizocilpine

mRNA	messenger RNA
NAc	nucleus accumbens
NMDA	N-methyl-D-aspartic acid
NRG-1	neuregulin 1
NVHL	neonatal ventral hippocampal lesion
PD	postnatal day
PET	positron emission tomography
PFC	prefrontal cortex
poly I:C	polyriboinosinic-polyribocytidilic acid
PPT	pedunculopontine tegmentum
PVDF	polyvinylidene fluoride
RNA	ribonucleic acid
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SNARE	Soluble NSF Attachment Protein Receptor
STN	subthalamic nucleus
SN	substantia nigra
SNAP 25	synaptosomal-associated protein 25
SNpc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
TBS-T	Tris-Buffered Saline and Tween 20
TNF-α	tumor necrosis factor-α
VGAT	vesicular GABA transporter

VGLUT	vesicular glutamate transporter
VMAT	vesicular monoamine transporter
VTA	ventral tegmental area

1. INTRODUCTION

1.1. Schizophrenia

Schizophrenia is a severe and life-long psychotic illness which has existed since the beginning of written history (Tamminga & Holcomb, 2005). It was, however, only at the end of the nineteenth century that schizophrenia was finally identified as a brain disorder as opposed to a disease of the mood (Tamminga & Kane, 1997). The biggest obstacle to the development of effective drugs devoid of side effects is our lack of understanding of this disease and its relevant molecular targets. To date, drug development has progressed serendipitously or by modifications to existing treatments (Tamminga et al., 2005). In order to design and develop fully effective treatments, drug discovery must be based on knowledge (Tamminga et al., 2005). This fuels the continuation of the search for the mechanisms and proteins involved in the pathology of schizophrenia.

1.1.1. Incidence and Prevalence

Schizophrenia is a debilitating mental disorder affecting approximately 1.1% of the world's population, amounting to 51 million people today. This mental illness remains a costly brain disorder to manage (Laruelle, Kegeles, & Abi-Dargham, 2003; Chen et al., 2004b; Mirnics, Middleton, Lewis, & Levitt, 2001; van Os & Kapur, 2009). Prognosis of schizophrenia was found significantly better in developing/rural, as opposed to developed/urban, areas of residence and birth (McGrath et al., 2004; Saha, Chant, Welham, & McGrath, 2005). Other studies estimate the prevalence of the disease to be

higher in low socio-economic groups and immigrant groups (McGrath et al., 2004; Roth, Lubin, Sodhi, & Kleinman, 2009; Saha et al., 2005). Interestingly, gender differences have also been found in schizophrenia. Men have an age of onset of about 15-25 years of age, while women show a later onset of 25-30 years of age, along with a smaller second and third peaks of incidence at 46-55 years of age and over 65 years of age, respectively (Leung & Chue, 2000). Not only does onset of disease occur later in women, a less severe course of disease development until menopause has been found when compared to men (Garcia-Segura, Azcoitia, & DonCarlos, 2001). The presence of estrogen is suggested to be protective against both the severity of symptoms and the timing of symptomatic onset in schizophrenia (Hafner, 2003; McGrath et al., 2004; Garcia-Segura et al., 2001). Furthermore, a greater degree of lateralization and the resulting lack of compensation in the male's brain may also place males at a higher risk towards disease development as compared to women (Leung et al., 2000; Garcia-Segura et al., 2001). Despite gender differences in disease onset and outcome, there is equal lifetime incidence between the sexes (Hafner, 2003). Another trend of symptom improvement is observed after 50 years of age in some patients, which could be attributed to either a less demanding period of life or a possible therapeutic effect of the aging process (Tamminga et al., 2005). Importantly, the aforementioned factors are a few of many contributing variables not independent of each other. Rather, the close interplay of many factors of susceptibility govern disease incidence, prevalence, and outcome. As a case in point, the weaker the patients' genetic or environmental predisposition, the stronger the ability of available estrogen expression levels to alter disease outcome (Hafner, 2003; Leung et al., 2000).

Exact mechanisms surrounding the perceived therapeutic effects of select trends in clinical populations are, however, not clearly understood as of yet and must be further explored in future studies.

1.1.2. Symptomology

Symptoms of schizophrenia are characterized by disturbances in a variety of behaviours and are categorized into three distinct symptom domains, namely the positive, negative, and cognitive symptoms (Laruelle et al., 2003; Chen et al., 2004b; Mirnics et al., 2001; van Os et al., 2009; Mathews & Muzina, 2007). Positive symptoms involve an excess of normal functions, such as hallucinations, delusions, and paranoia (McGrath et al., 2004; Tamminga et al., 2005; Roth et al., 2009). Negative symptoms comprise of deficits in functions, such as a lack of pleasure in activities (anhedonia), social withdrawal, and a lack of motivation (McGrath et al., 2004; Roth et al., 2009; Tamminga et al., 2005). Cognitive symptoms include impairments in memory, deficits in attention, disturbances in executive functioning, and disorganized thoughts (McGrath et al., 2004; Roth et al., 2009; Tamminga et al., 2005). Cognitive symptoms have been identified as a "core prodromal" symptom of the disease, often manifesting preceding the other two classes of symptoms (Lencz et al., 2006). Differing classes of symptoms can co-exist together, or dominate separately and independently of each other (Tamminga et al., 2005). Characteristic of schizophrenia is a quick onset of clinical symptoms typically during adolescence or early adulthood, with peak onset in the second and third decades of life (Tamminga et al., 2005; McGrath et al., 2004). Rare cases of childhood-onset

schizophrenia occur prior 15 years of age, at an incidence rate of 50 times less than classic schizophrenia (McGrath et al., 2004; Tamminga et al., 2005; Leung et al., 2000). Schizophrenia is a disease episodic in nature and can have stagnant periods of inactivity in between episodes (Tamminga et al., 2005). In most cases, the disease initiates with fast deterioration of psychosocial function within the first few years, and is followed by a plateau of symptomology during the later course of the disease (Tamminga et al., 2005).

Schizophrenia is a disease of heterogeneity, which is evidenced by separate domains of symptoms arising from variable pathological mechanisms and the lack of an effective gold standard treatment to appropriately alleviate symptoms (Tamminga et al., 2005). Despite the availability of drug treatments to improve the outcome of disease, a significant portion of afflicted patients still suffer from the burden of symptoms and lifelong psychosocial impairments (Tamminga et al., 2005). Surveys find 10-20% of patients recover to pre-illness function, 15-33% of patients remain treatment-resistant, and the remaining of patients continue to suffer ongoing psychotic, affective, and cognitive impairments, despite ongoing medications (Tamminga et al., 2005).

1.2. Hypotheses of Schizophrenia

Schizophrenia has been thought to arise from a combination of genetic, environmental, and developmental factors (Tseng, Chambers, & Lipska, 2009). These various theories are not necessarily incompatible, but may likely operate in a cumulative or synergistic manner to impact the final outcome of disease (Leung et al., 2000). An individual's genetic profile may moderate the effects the environment has on

physiological pathways. On the contrary, the environment may also influence the expression of risk genes that would be causal to the manifestation of psychosis (van Os, Rutten, & Poulton, 2008). Multiple factors come into play, and may act in an additive manner to determine susceptibility of an individual (Tamminga et al., 2005).

1.2.1. Genetic Hypothesis of Schizophrenia

Genetics plays a major role in one's predisposition towards disease manifestation (Tamminga et al., 2005). Without direct molecular markers, indirect measures of genetic risk and transmission of vulnerability towards disease development can be gathered using family, twin and adoption studies (van Os et al., 2008; Roth et al., 2009; Rietkerk et al., 2008). As the degree of relatedness to the affected individual increases, the risk for developing schizophrenia also increases. The general rate of disease prevalence is 1.1% in the general population, but this rate increases to 8-10% in persons with a sibling affected by the disease, 12% in persons with a parent affected by the disease, 15% in dizygotic twins, and 40-53% in monozygotic twins (Tamminga et al., 2005; van Os et al., 2008; Roth et al., 2009). Examples provide strong evidence for the familial component of schizophrenia (Roth et al., 2009).

There are no known molecular markers of the disease, but genes of susceptibility have been found linked to chromosomal regions 8p, 22q, 2,3, 5q, 6p, 11q, 13q, and 20p (Tamminga et al., 2005; Roth et al., 2009; Tan et al., 2014). Genes associated with the aforementioned chromosomal regions include dysbindin (DTNBP-1), neuregulin (NRG1), proline dehydrogenase, and D-amino-acid oxidase, amongst many others

(Stefansson et al., 2002; Schwab et al., 2003; Chumakov et al., 2002; Chowdari et al., 2002; Liu et al., 2002; Shifman et al., 2002). More than 50% of genes reported to be associated with schizophrenia, including brain-derived neurotrophic factor (BDNF), metabotropic glutamate receptor 3, DTNBP-I, and AKT-1, are subject to regulation by environmental insults such as hypoxia (van Os et al., 2008; Roth et al., 2009).

Moreover, Mirnics *et al*. further indicated that genes most altered in schizophrenia are involved in or possess presynaptic secretory function, including regulator of G protein signalling 4, RAB-3, N-ethylmaleimide sensitive fusion protein, and synapsin II (Mirnics et al., 2001; Muller, Pym, Tong, & Davis, 2011; Tan et al., 2014; Sawa & Snyder, 2002). Schizophrenia is considered a disease of synaptic disconnection and disorganization; a defect in synaptic genes may impair neuron signalling, alter synapse formation, and ultimately influence the onset of mental illnesses (McGlashan & Hoffman, 2000; Mirnics et al., 2001).

1.2.2. Environmental Hypothesis of Schizophrenia

Environmental influences can be persuasive and impact disease outcome based on predisposing genetic makeup. Children from schizophrenic parents raised by normal parents had a 18.8% risk of disease development while those from normal parents raised by schizophrenic parents had a 19.7% risk of disease development (Roth et al., 2009). Moreover, a degree of discordance exists between monozygotic twins with 100% genetic identity. Thus, the likelihood of environmental influences and epigenetic changes must underscore the genetic vulnerability towards production of psychosis (Roth et al., 2009;

van Os et al., 2008). An individual's vulnerable genotype can be further exacerbated by environmental factors. For instance, valine and not methionine, allele carriers of a functional polymorphism in catechol-*O*-methyl transferase portray increased sensitivity towards the psychotogenic effects of drugs of abuse or stress (Roth et al., 2009; van Os et al., 2009; van Os et al., 2008). Environmental components which influence the outcome of disease may come in the form of psychosocial, biological, or physical factors which an individual encounters from the time of conception, through development, birth, and maturation into adulthood (Tsuang, 2000).

The largest environmental risk for schizophrenia has been reported to be obstetric complications, such as preeclampsia and perinatal brain damage (Roth et al., 2009; Tamminga et al., 2005; Petronis, Paterson, & Kennedy, 1999). An increase in births of patients with schizophrenia has also been found during the winter months of the year, which is attributed to higher rates of infection during winter (Petronis et al., 1999; Tsuang, 2000; Tamminga et al., 2005; Roth et al., 2009). Moreover, as previously alluded to, urban births have at least a two-fold increase in risk towards developing schizophrenia (Tsuang, 2000). The stress in an urban environment with a higher population density can include excessive noise, pollution, crime, divorce, and other negative social factors which can precipitate schizophrenia in genetically vulnerable individuals (Tsuang, 2000).

Interestingly, comorbidity of substance abuse and schizophrenia is found in as high as 59% of affected patients (Cantor-Graae, Nordstrom, & McNeil, 2001; Blanchard, Brown, Horan, & Sherwood, 2000). Patients with schizophrenia suffer addictions to psychostimulants at two to four fold times greater than the general population (Tseng et

al., 2009). Psychostimulants abused include cannabis, alcohol, nicotine, amphetamine, phencyclidine (PCP), and cocaine (Cantor-Graae et al., 2001; Tseng et al., 2009; Roth et al., 2009; Blanchard et al., 2000). The use of stimulants can result in poorer outcomes in patients, including greater rates of treatment refractories, treatment non-compliance, hospitalizations, homelessness, and more (Tseng et al., 2009). Co-morbidity of substance abuse and schizophrenia could either be due to a heightened vulnerability towards psychoactive substances in individuals with increased susceptibility (i.e. a genetic predisposition or altered brain reward circuit dysfunction) or the cumulative effects of matching risk factors for both schizophrenia and substance abuse (i.e. poverty, poor education, poor social relations) (Cantor-Graae et al., 2001; Green, Drake, Brunette, & Noordsy, 2007; Blanchard et al., 2000). The direction of causality remains controversial as it is presently unclear if substance abuse is an independent risk factor or if substance abuse precipitates illness onset in vulnerable individuals (Cantor-Graae et al., 2001).

Given the close interaction between an individual's genes and the environment, molecular mechanisms mediating the interplay between the two are likely to have significant impact on the onset of schizophrenia (Roth et al., 2009). The environment can exert its influence on the activity and expression of genes via epigenetic mechanisms (Roth et al., 2009). Unlike stable genetic sequences, epigenetic changes are only partially stable in its contribution to both gene activity and the dynamic state of the central nervous system (CNS) system throughout one's lifetime (Petronis et al., 1999). Epigenetic mechanisms through DNA methylation and histone modifications can work to either suppress or activate gene transcription (Roth et al., 2009). Cytosines followed by

guanines form islands of CpG nucleotide sequences, which are typically found within the gene's regulatory regions (Roth et al., 2009). Methylated cytosines within these islands recruit methyl-DNA binding proteins, which then employ histone deacetylases to remove acetyl groups from the histone proteins (Roth et al., 2009). These processes work to compact chromatin structure, limiting accessibility of transcription machinery and thus suppressing gene transcription and activation (Roth et al., 2009). DNA methylation and histone modifications are not restricted to the suppression of genes, and can also activate gene expression. Social experiences during the first postnatal week have been found to cause lasting changes in DNA methylation and gene expression well into adulthood (Roth et al., 2009). Epigenetic mechanisms can also involve non-coding RNA such as intracellular microRNAs (miRNA) which are expressed naturally in the brain. miRNAs modulate the natural inhibition of gene expression and has been shown to play a role in disease generation. Evidence indicate several miRNAs altered in the PFC of patients afflicted with schizophrenia (Miller et al., 2012; Perkins et al., 2007). miRNA-219 has specifically been found to modulate NMDA-mediated hypofunction, which is a phenomenon commonly associated with symptoms of schizophrenia (Kocerha et al., 2009). Epigenetic mechanisms can affect synaptic plasticity, cell differentiation, and genomic imprinting, amongst others (Roth et al., 2009; Petronis et al., 1999). Epigenetic changes can also influence genes involved in major neurotransmitter systems in schizophrenia, including the GABAergic, glutamatergic, serotonergic, and dopaminergic systems (Roth et al., 2009). Further, epigenetic changes can be inherited by future generations (Roth et al., 2009). Epigenetic changes may contribute to the aberrant

regulation of associated genes in schizophrenia, and are useful for studying the interaction of the environment on existing genotypes.

It is important to note that environmental risks on their own are less likely to cause the production of schizophrenia, and environmental factors must be supplemented with a genetic disposition in order for the disorder to develop (Tsuang, 2000). Likewise, despite being an influential factor, genetics alone do not determine disease outcome, and the influence of the environment must not be undermined. Interventions to reduce environmental risk factors in genetically susceptible children may prove to be an effective method to alter disease outcome (Tsuang, 2000).

1.2.3. Neurodevelopmental Hypothesis of Schizophrenia

The neurodevelopmental hypothesis of schizophrenia was first proposed by D.R. Weinberger in 1987. This neurodevelopmental hypothesis postulates that pathogenic processes occur early during development and long before the illness is clinically expressed (Meyer & Simpson, 1997; Lencz et al., 2006). Subtle alterations to specific neurons and circuits during early development are sufficient to confer vulnerability in an individual, ultimately leading to the malfunction of neural processes and the full-blown clinical manifestation of schizophrenia by adolescence or early adulthood (Lieberman et al., 2001). Features of the neurological sequelae only manifest as full-blown psychosis when the affected neural systems mature and interact with the developmentally-disturbed brain region of interest (Meyer et al., 1997; McGrath et al., 2004). Not surprisingly, genes found implicated in schizophrenia, such as NRG-1, dysbindin, and synapsins, are highly

involved in early developmental processes of synaptic plasticity and the sound functioning of neurons and glial cells (McGrath, Feron, Burne, Mackay-Sim, & Eyles, 2003).

Brain development is a continuing process in which subtle perturbations from its normal course of growth can influence the production of abnormalities and consequent mental illness (Marenco & Weinberger, 2000). To date, pathogenic mechanisms and risk factors surrounding the effect of prenatal or perinatal disturbances on the production of symptoms remain obscure, as does the specificity of infection or insult (Marenco et al., 2000; Rapoport, Giedd, & Gogtay, 2012). Neurodevelopmental disturbances are thought to be risk factors include early life exposures to viral infections, obstetric complications, and/or a less ideal birthing environment (i.e. winter, urban location). Various specific and non-specific factors affecting brain development can increase the risk for psychosis later on in life (Marenco et al., 2000). Obstetric complications, including shorter gestation periods and preeclampsia, or non-specific disturbances such as head trauma, may confer a higher risk (Marenco et al., 2000). Delivery complications, such as preeclampsia, can result in fetal hypoxia and brain damage, and lead to a nine-fold increase in fetal risk for schizophrenia (Tsuang, 2000; Roth et al., 2009; Tamminga et al., 2005; Petronis et al., 1999). Hypoxia-related obstetric complications result in more than five-fold risk to developing schizophrenia than those with no hypoxia-related obstetric complications (Cannon, Jones, & Murray, 2002). Influenza, Coxsackie B, measles, polio, and varicella are viruses implicated with development of schizophrenia, of which the peak vulnerability period was found to be during the second trimester of the pregnancy

(Tsuang, 2000; Ozawa et al., 2006; Patterson, 2009). Viral infections can cause abnormalities in brain development, resulting in fetal death, premature delivery, low birth weight, and brain damage, which influence disease outcome (Tsuang, 2000; Petronis et al., 1999; Tamminga et al., 2005; Roth et al., 2009). Other prenatal and perinatal risks for schizophrenia include Rhesus incompatibility, unwanted pregnancy, maternal malnutrition (in first trimester), and maternal influenza (in second trimester) (Tsuang, 2000; Petronis et al., 1999; Tamminga et al., 2005; Roth et al., 2009). This notion of adult-onset schizophrenia having its roots in development is further supported by a number of primate and rodent studies showing induced neonatal brain lesions having delayed effects on the development of abnormal behaviour (Marenco et al., 2000; Owen, O'Donovan, Thapar, & Craddock, 2011).

Two basic types of neurodevelopmental theories exist for schizophrenia, namely an early neurodevelopmental theory and a late neurodevelopmental theory (Leung et al., 2000). In the early neurodevelopmental model, brain aberrations occur during the prenatal and neonatal period. Brain interruptions can come in the form of viral infections and obstetric complications, amongst many other environmental factors (Leung et al., 2000). A failure to compensate for the induced abnormalities could lead to the development of psychosis and other related symptoms in early adulthood when the affected brain regions reach maturity (Leung et al., 2000). On the contrary, in the late neurodevelopmental model, brain aberrations occur later in life, particularly during adolescence. During normal brain development, there is a dramatic increase in synapses in the first few years of life, which is then followed by a period of pruning in adolescence

in which unused synapses are eliminated (Leung et al., 2000; Tsuang, 2000; McGrath et al., 2003). Abnormal over- or under- pruning of synapses in the prefrontal cortex (PFC) or subcortical regions of the brain, respectively, can create a foundation to the production of psychosis and related symptoms of schizophrenia (Leung et al., 2000). Evidence has also been provided for the merging of these two developmental theories, such that pathogenic life events do not necessarily have to only result from a one-time "hit" early in life, but could involve a combination of multiple risk factors and additional “hits” over one's lifetime (McGrath et al., 2003). Additional “hits”, such as drug abuse, excessive synaptic pruning during adolescence or a failure to treat early symptoms, can trigger full-blown manifestation of symptoms and lead to poorer disease outcomes (McGrath et al., 2003).

1.2.3.1. Prefrontal Cortex

Observations from clinical, neuroimaging, and anatomical studies have focused attention on the PFC as the major alteration site in schizophrenia (Mirnics et al., 2001; Dyck et al., 2009; Dyck, Beyaert, Ferro, & Mishra, 2011). The period of adolescence and adulthood has been observed to be a stage of particular vulnerability to the expression of psychotic and non-psychotic symptoms pertinent to schizophrenia (Marenco et al., 2000). This phenomenon of late symptom manifestation can be explained by the order of brain development; the trimming of relevant synapses in the brain starts from the posterior regions of the brain and move anteriorly, such that higher-ordered brain regions like the PFC become the last of the brain regions to be pruned and sculpted by neuronal activity

(Gogtay et al., 2004). As the longest to mature and tardiest to develop amongst the brain regions, the PFC only reaches its full maturity as early as mid-20s to 30s in humans (**Figure 1**) (Kolb et al., 2012). Synaptic irregularities induced from birth, within the PFC may remain "offline" during brain development up until the function of the brain region is summoned "online" during a time period of molecular changes and neuronal pruning which, in this case, aligns with the period of adolescence and adulthood in humans (**Figure 1**) (Kolb et al., 2012; Marenco et al., 2000). This late maturation of the PFC may explain the delayed production of clinical symptoms within this neurodevelopmental model of schizophrenia (Marenco et al., 2000). Theoretically, disturbances from critical stages in development should result in static pathology until the later stages of adulthood when the clinical symptoms come "online" with the maturation of the frontal cortex (Marenco et al., 2000). However, despite this common conception of schizophrenia being an "adult-onset" disorder, many patients also display premorbid neurological and behavioural abnormalities, including those in motor behavior, cognition, and social relatedness, prior the onset of clinical illness in as early as childhood and adolescence (Marenco et al., 2000; Diamond, 2000).

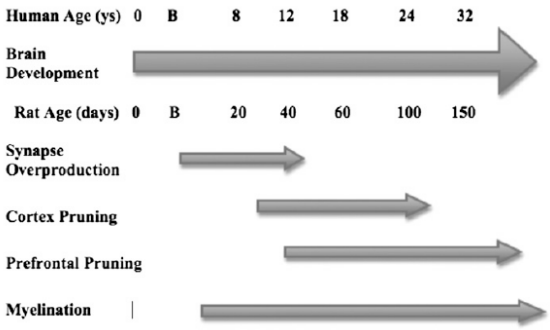


Figure 1: Timeline for the development and synaptic pruning of the PFC, as reviewed by Kolb *et al*. (Kolb et al., 2012). PFC pruning is cited to initiate at 8 years of age and reach full maturity at 32 years of age in humans; this is equivalent to a rat's pruning initiating at 30 days of life and reaching full maturity at 150 days of life. At this stage of pruning and brain maturation, abnormalities in the PFC become prominent through the manifestation of symptoms owing to the summoning of this brain region and its functions.

1.3. Pathophysiology

With interplay of polygenetic susceptibility, epigenetic changes and environmental factors that can predispose an individual to developing schizophrenia, there must be interplay in the multiple circuits factoring into both the expression of the diseased phenotype and treatment of schizophrenia (Ferreira, Han, Greengard, & Kosik, 1995; Guidotti et al., 2000; Laruelle et al., 2003). Evidence suggests the dysregulation and interaction of several neurotransmitter systems, amongst which include the dopamine, glutamate and GABA systems. A holistic approach of studying this disorder will lend to the development of new and effective prevention strategies targeting schizophrenia (Carlsson et al., 2001).

1.3.1. Dopamine

The dopamine hypothesis has been the most widely accepted hypothesis for schizophrenia (Carlsson et al., 2001). This hypothesis attributes overactive dopamine systems in the subcortical regions in patients with schizophrenia (Featherstone, Kapur, & Fletcher, 2007; Peleg-Raibstein, Knuesel, & Feldon, 2008). Evidence for this has been provided by use of pharmacological agents, such dopamine agonists (e.g. amphetamine), which induce schizophrenia-like symptoms in humans. These dopaminergic pharmacological agents are also common inducers of schizophrenia-like behaviour in preclinical animal models. In addition, Single Photon Emission Computed Tomography and Positron Emission Tomography (PET) studies have shown that patients with schizophrenia show elevated synthesis and release of dopamine in the basal ganglia

(Carlsson et al., 2001; Carlsson et al., 2001). This elevated dopamine release, along with the increased number of dopamine D2 receptors in the high affinity state ($D2^{High}$), correlate with the manifestation of the positive symptoms observed in schizophrenia (Featherstone et al., 2007; Petronis et al., 1999; Seeman, 2011). Interestingly, the potency and efficacy of current antipsychotic drugs to treat symptoms of schizophrenia is directly correlated to their occupancy at the dopamine D2 receptor, substantiating the role of hyperdopaminergic activity in mechanisms underlying this disorder (**Figure 3**) (Featherstone et al., 2007; Laruelle et al., 2003; Carlsson et al., 2001; Kapur, Zipursky, Jones, Remington, & Houle, 2000; Petronis et al., 1999; Seeman, 2011). In addition, there is also a deficit in dopamine activity in the PFC (Featherstone et al., 2007). This hypofrontality is correlated with low levels of the dopamine metabolite, homovanillic acid (Featherstone et al., 2007). The under-stimulation of the dopamine D_1 receptors and low dopamine activity in the PFC has been suggested to correlate with cognitive impairment and poor performance in tasks involving working memory seen in patients with schizophrenia (Thierry, Gioanni, Degenetais, & Glowinski, 2000; Laruelle et al., 2003; Tamminga et al., 2005). The revisited dopamine hypothesis attributes excess dopaminergic activity in the striatum to the positive symptoms, as well as reduced dopaminergic signalling in the PFC to both the negative and cognitive symptoms of the disease (**Figure 2B**) (Featherstone et al., 2007).

1.3.2. Glutamate

There is a growing body of evidence suggesting the involvement of glutamate in schizophrenia, of which theory attributes hypoglutamatergic activity in the cortical regions of the brain. Use of pharmacological challenges, such as N-methyl-D-aspartic acid (NMDA) receptor antagonists, in both humans and animals are seen to induce behavioural and cognitive deficits which closely represent symptoms seen in schizophrenia (Tamminga et al., 2005; Goff & Coyle, 2001). These NMDA antagonists not only induce cortical hypoglutamatergic activity but also enhance the spontaneous release of subcortical dopamine during a challenge, comparably to dopamine agonist-induced preclinical models of schizophrenia (Carlsson et al., 2001). In fact, NMDA antagonists, PCP and ketamine, induce more profound positive and negative deficits representative of schizophrenia than those induced by dopamine agonists (i.e. amphetamines) (Carlsson et al., 2001). NMDA receptor-deficient mice exhibit schizophrenia-like behaviour, including hyperactivity, stereotypy, and deficits in social interaction. These behavioural abnormalities are attenuated by treatment with antipsychotic drugs like haloperidol and clozapine (Südhof et al., 1989). Conversely, increasing NMDA receptor activity with NMDA agonists like glycine and D-serine can alleviate symptoms in patients with schizophrenia (Laruelle et al., 2003; Greengard, Valtorta, Czernik, & Benfenati, 1993). Evidence substantiates the glutamate hypothesis to better represent the mechanisms surrounding this disorder and provides rationale for the importance of studying glutamatergic aberrations in schizophrenia (**Figure 3**). Evidence for both hypoglutamatergic and hyperdopaminergic activity is not exclusive, but rather

behaves in a complementary manner. Sustained NMDA hypofunction has been found to induce a reduction in mesocortical dopamine transmission and subsequent increase in subcortical mesolimbic dopamine activity, trends of which are consistent with the dopamine hypothesis of schizophrenia (**Figure 2B, 4B**) (Laruelle et al., 2003). Synaptic alterations within the PFC may primarily result in sustained dysfunction of glutamate neurotransmission and cause subsequent secondary abnormalities in dopamine transmission (subcortical dopamine hyperactivity and cortical dopamine hypoactivity) (**Figures 2A-B, 4A-B**) (Laruelle et al., 2003).

1.3.3. GABA

Studies-to-date are starting to take a more multi-factorial approach by involving interactions and aberrations of several neurotransmitter systems including that of glutamate, GABA, and serotonin (Carlsson et al., 2001). The dopamine hypothesis associates hyperdopaminergic activity in the striatum to account for positive symptoms, while hypodopaminergic activity in the cortical regions to be responsible for negative and cognitive symptoms (Bogen et al., 2006; Hilfiker et al., 1999). The glutamate hypothesis implicates glutamate hypofunctionality in the cortical regions of the brain to account for both negative and neurocognitive symptoms of schizophrenia (Kao et al., 1999; Hilfiker et al., 1998). Both glutamatergic and dopamine projections all converge on the dendritic spines of GABAergic medium spiny neurons in the striatum (Laruelle et al., 2003). This point of convergence is important for the modulatory effects of dopamine on glutamatergic transmission, and vice versa (Carlsson et al., 2001).

Over the years, the GABA hypothesis has remained in the shadow of the more dominant dopamine and glutamate hypotheses, but increasing support has been gained for this GABA-deficient theory from clinical and preclinical experiments (Kalkman & Loetscher, 2003). GABAergic neurons form the largest population of neuronal cells and is the major inhibitory neurotransmitter in the brain (Carlsson et al., 2001; Kalkman et al., 2003). Moreover, cortical glutamate hypoactivity in schizophrenia is said to occur on GABA interneurons (Carlsson et al., 2001). A dysregulation in GABAergic neurotransmission has been observed with a reduction in GABA-synthesizing enzymes reported in patients with schizophrenia (Pieribone et al., 1995; Ferreira, Kosik, Greengard, & Han, 1994). Additionally, a reduction in GABA neuron density has been observed in the PFC and limbic regions of patients with schizophrenia (Carlsson et al., 2001).

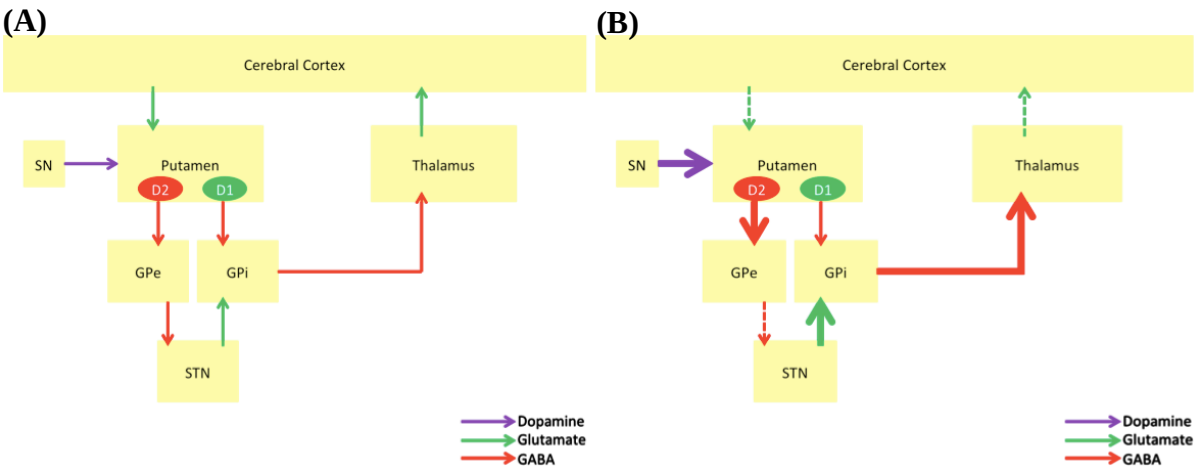
Synthesis of GABA is regulated by the enzyme glutamic acid decarboxylase (GAD), which is present as two independently-regulated isoenzymes, GAD_{65} and GAD_{67} (Kalkman et al., 2003). GAD_{67} is reported to have a more pronounced effect on brain GABA synthesis than its smaller isoenzyme, GAD_{65} (Kalkman et al., 2003). Specifically, there is a decrease in expression levels of isoenzyme, GAD_{67}, in the dorsolateral PFC, hippocampus, temporal cortex, cerebellum, and caudate nuclei of patients with schizophrenia (Kalkman et al., 2003). A reduction in GAD expression levels indicate reduced GABA expression levels, and thus a reduction in GABAergic inhibitory control on neurons (Kalkman et al., 2003). Notably, changes in GAD_{67} expression levels are not limited to schizophrenia, and have been found in other psychotic disorders including

bipolar disease (Kalkman et al., 2003). Interestingly, animal models of schizophrenia indicate diminished expression levels of GAD_{67} following both chronic stimulation of dopamine D_2 receptors and short-term blockade of NMDA receptors (Kalkman et al., 2003). Conversely, treatment with dopamine D_2 receptor antagonists enhances expression levels of GAD_{67} (Kalkman et al., 2003). The role of GABA must not be underestimated in the pathology and treatment of schizophrenia.

1.3.4. Serotonin

Evidence for the alteration of the serotonergic system in schizophrenia first emerged with the finding that use of lysergic acid diethylamide (LSD), a potent 5-serotonin-2 (5-HT_2) receptor agonist, resulted in hallucinations and delusions commonly found in patients with schizophrenia (Roth et al., 2009). LSD did not, however, induce the production of negative symptoms such as social withdrawal and blunted affect (Roth et al., 2009). Within the 3 subtypes of 5-HT_2 receptors, variation of the gene specifically encoding for the 5-HT_{2A} receptor was found to be associated with schizophrenia (Roth et al., 2009). Moreover, the new generation of atypical antipsychotic drugs have all been postulated to mediate their effective antipsychotic effect and decreased rates of extrapyramidal side effects (EPS) through 5-HT-receptor based mechanisms (Meltzer, Li, Kaneda, & Ichikawa, 2003). Specifically, potent 5-HT_{2A} receptor antagonism, along with weak dopamine D_2 receptor antagonism are principal pharmacological features which distinguish second generation atypical from first generation typical antipsychotic drugs (Meltzer et al., 2003). However, due to the scope of this study, the serotonergic system

was not explored in detailed. Further investigations must examine the involvement of this neurotransmitter system in this newly developed animal model of schizophrenia.



Figures 2A-B: Oversimplied drawings of the direct (D_1; stimulatory) and indirect (D_2; inhibitory) pathways. (**A**) Diagram illustrates both these pathways in a normal brain. (**B**) Diagram illustratrates the hypothesized neurocircuitry in a schizophrenic brain, where there is an increase in dopaminergic activity (purple arrow) projecting into the subcortical regions (putamen/striatum) as well as increased dopamine D_2 receptor activity (bolded red arrow). This increase in dopaminergic activity causes an increase in GABA output to the globus pallidus external (GPe). Increased inhibition caused by GABA to the GPe results in decreased in GABA output to the subthalamic nucleus (STN), and subsequent increase in glutamatergic output from the STN into the globus pallidus internal (GPi). Increased glutamate activation into the GPi results in increased GABA output from the GPi into the thalamus, and decreased glutamatergic transmission back into the cortex. In schizophrenia, overstimulation of the dopamine D_2 receptor (increased dopaminergic activity in the striatum/putamen) ties in closely with a decrease in glutamatergic transmission within the PFC. Not displayed in this figure is decreased dopaminergic activity within the PFC (**See Figure 5**). Figures are modified from Carlsson *et al.*, Laruelle *et al.*, and are heavily supplemented with other primary studies and extensive reviews described throughout this thesis (Carlsson et al., 2001; Laruelle et al., 2003; Carr & Sesack, 2000). NB: Direct dopamine D_1 pathway activation – stimulatory; Indirect dopamine D_2 pathway – inhibitory. GPe – globus pallidus external; GPi – globlus pallidus internal; SN – substantia nigra; STN – subthalamic nucleus.

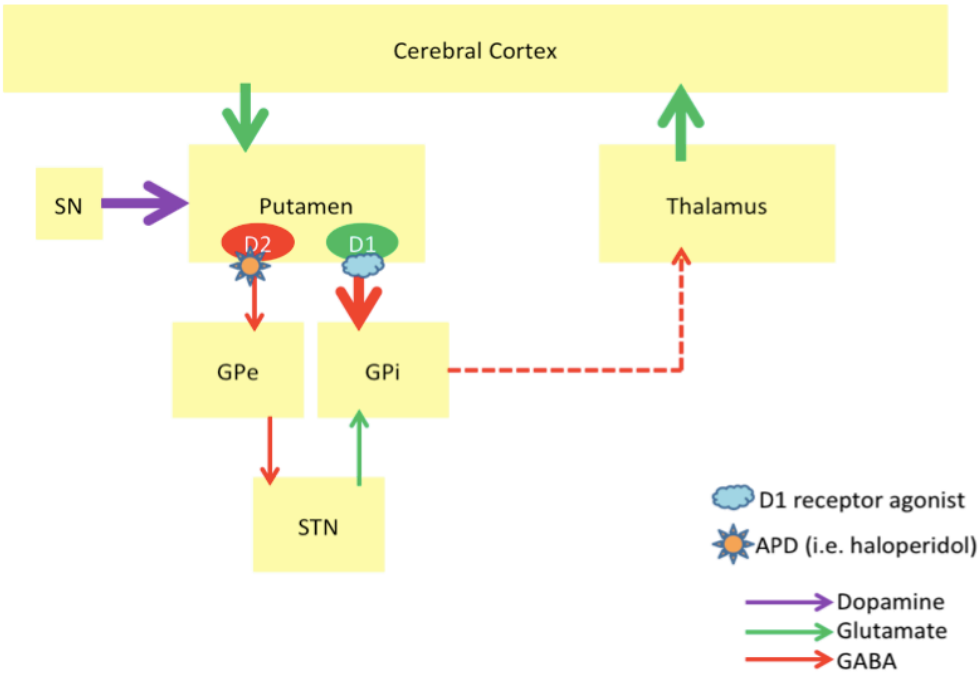


Figure 3: Proposed therapeutic mechanisms of current antipsychotic drugs. Current antipsychotic drugs primarily target the dopamine D_2 receptor, thereby antagonizing hyperdopaminergic activity in the striatum/putamen. Antagonists of the D_2 receptor (i.e. haloperidol) or D_1 receptor agonists can potentially facilitate an increase in downstream glutamatergic transmission into the PFC, and cause a normalization of the neurocircuitry pathways implicated in schizophrenia (Paine, Neve, & Carlezon, Jr., 2009). Not displayed in this figure is decreased dopaminergic activity in the schizophrenic PFC (**See Figure 5**). Figures are modified from Carlsson *et al.*, Laruelle *et al.*, and are heavily supplemented with other primary studies and extensive reviews described throughout this thesis (Carlsson et al., 2001; Laruelle et al., 2003; Carr et al., 2000). NB: Direct dopamine D_1 pathway activation – stimulatory; Indirect dopamine D_2 pathway – inhibitory. GPe – globus pallidus external; GPi – globlus pallidus internal; SN – substantia nigra; STN – subthalamic nucleus.

1.4. Neurocircuitry

Synaptic contacts and projections between regions of the brain are not randomly distributed, but are rather designed to target specific cell populations (Carr et al., 2000). The percentage of afferent and efferent neurons tethering between select brain regions do not necessarily determine the probability of receiving synaptic contacts. Rather, the functional influence of each individual pathway is determined by specific targeting of select neuronal populations (Carr et al., 2000).

1.4.1. Prefrontal Cortex-Ventral Tegmental Area Projections

Dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and PFC are important for motivation, reward, and cognitive functions (Carr et al., 2000). Excitatory glutamatergic projections from the PFC synapse onto dopamine and GABA neurons in the VTA (Carr et al., 2000). Stimulation of this principal glutamatergic input from the PFC to the VTA causes an increase in dopamine neuron firing while blockade of glutamate receptors within the PFC was conversely found to suppress dopaminergic activity within the VTA (Carr et al., 2000). Stimulation of glutamatergic neurons within the PFC also increased dopamine levels within the PFC, whereas antagonism of PFC glutamate function suppressed dopamine activity within the PFC (Carr et al., 2000). Within the VTA, one-third of PFC glutamatergic projections may selectively synapse onto the GABA neurons which project to the NAc while another one-third of PFC projections synapse on the dopamine neurons which project back into the PFC (Carr et al., 2000). Additionally, the PFC likely mediates an indirect glutamatergic

effect on the VTA through the pedunculopontine tegmentum (PPT); these excitatory projections from the PFC indirectly affect the dopamine neurons in the VTA (**Figure 4**) (Carr et al., 2000). Excitatory PFC projections onto the GABA neurons in the VTA can indirectly regulate the mesoaccumbens dopaminergic projections (Carr et al., 2000). Functional deficits within the PFC can result in the loss of inhibition of the mesostriatal dopamine neurons, contributing to the pathophysiology of schizophrenia (**Figure 4B**) (Carr et al., 2000). Additional non-PFC innervated GABAergic neurons project from the VTA into the PFC, comprising 58% of all mesofrontal neurons (Carr et al., 2000). These VTA-PFC GABA projections synapse on both local GABA circuit neurons and pyramidal cells within the PFC (Carr et al., 2000) (**Figure 4A-B**).

1.4.2. Prefrontal Cortex-Hippocampus Projections

Both the PFC and hippocampus are crucial brain regions for memory processes and are functionally and anatomically linked (Thierry et al., 2000). The PFC is involved in higher cognitive functions, including working memory and goal-directed behavior, while the hippocampus is involved in declarative memory and the encoding and formation of memories (Thierry et al., 2000). The NAc is also a region of the ventral striatum implicated in goal-directed behavior. The PFC receives dopaminergic projections from the VTA and glutamatergic projections from the hippocampus (Thierry et al., 2000; Jay, Glowinski, & Thierry, 1995). With the exception of the hippocampus and striatum, most of the prefrontal cortical connections with other cortical and subcortical regions of the brain are reciprocal (**Figure 4**) (Thierry et al., 2000). Both the

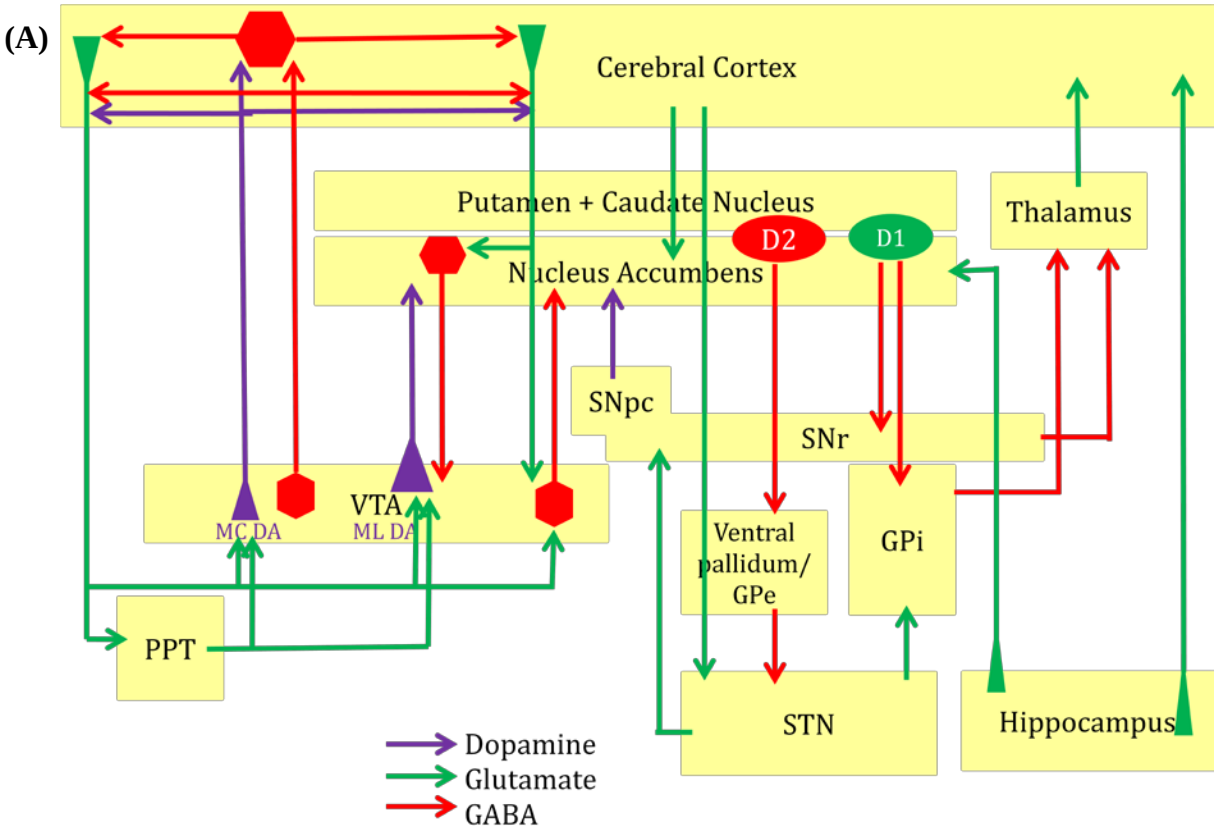
PFC and the dopamine innervations into this region are particularly important for cognitive, affective, motivational, and emotional behaviours (Thierry et al., 2000; Jay et al., 1995). Excitation of the PFC can be attributed to glutamatergic projections from the hippocampus, which can be inhibited by the activation of ascending mesocortical dopaminergic neurons (Jay et al., 1995). This mesocortical inhibition can be via a direct dopaminergic modulation on pyramidal cells or via an indirect mechanism involving GABAergic interneurons (**Figure 4**) (Jay et al., 1995). Long-term potentiation (LTP) has been observed following high frequency stimulation of the hippocampus (Jay et al., 1995). Subsequent excitation of the NAc and VTA can result from hippocampal-stimulated PFC neurons synapsing on the spiny neurons of the NAc and on the dopamine neurons of the VTA (**Figure 4**) (Jay et al., 1995). The PFC and dopaminergic systems have long been implicated in mental illnesses. Dopamine innervation from the VTA into the NAc is highly involved in the sensitization process induced by repeated administration of drugs of abuse (Jay et al., 1995). Although the role of hippocampus has not been clearly defined in its contribution towards symptom development in schizophrenia, a reduction in hippocampal volume and decreased density of parvalbumin cells can contribute to altered hippocampal-PFC-VTA and hippocampal-PFC-NAc circuits, further adding insult to a dysfunctional mesocortical and mesostriatal dopamine system (**Figure 4B**) (Jay et al., 1995; Zhang & Reynolds, 2002; Harrison, 2004).

1.4.3. Basal Ganglia Circuits

The prelimbic/medial orbital areas of the PFC projects glutamatergic excitatory inputs into the core of the NAc, which then cycles through either direct or indirect pathways of the basal ganglia circuit (Thierry et al., 2000). In the *direct* pathway, the PFC sends glutamatergic projections into the core of the NAc, which projects inhibitory projections into the dorsomedial portion of the substantia nigra pars reticulata (SNr) (Thierry et al., 2000). The SNr sends inhibitory projections into the mediodorsal thalamic and subsequent excitatory projections back into the PFC (Thierry et al., 2000). In the central physiology of the motor circuits of the basal ganglia, the activation of the direct pathway through the inhibition of tonically active GABAergic neurons in the SNr leads to the disinhibition of neurons in the thalamus and subsequent frontal cortex excitation (Thierry et al., 2000). The medial STN also receives excitatory input from the PFC, which allows the PFC excitatory control of the SNr (**Figure 4A-B**) (Thierry et al., 2000).

In the *indirect* pathway, prefrontal cortical activation of the NAc causes the activation of the GABAergic neurons in the ventral pallidum, which result in the inhibition of tonically active GABAergic neurons projecting into the subthalamic nucleus (STN) (Thierry et al., 2000). This results in increased excitatory glutamatergic neurons projecting out of the STN, and an activation of medial SNr output cells (Thierry et al., 2000). This leads to an inhibition of the thalamus with increased GABA input and subsequent decrease in excitability in the PFC (Thierry et al., 2000). The indirect pathway shapes the processes of the direct pathway by inhibiting competing movements (**Figure 4**). An imbalance between the direct striato-nigral and indirect striato-

subthalamic pathways is responsible for movement disorders such as dyskinesia, akinesia, and perturbs prefrontal cortical functions in cognition and memory (Thierry et al., 2000).


(A)
Cerebral Cortex
Putamen + Caudate Nucleus
Nucleus Accumbens
D2
D1
Thalamus
SNpc
SNr
VTA
MC DA
ML DA
Ventral pallidum/ GPe
GPi
PPT
STN
Hippocampus
Dopamine
Glutamate
GABA

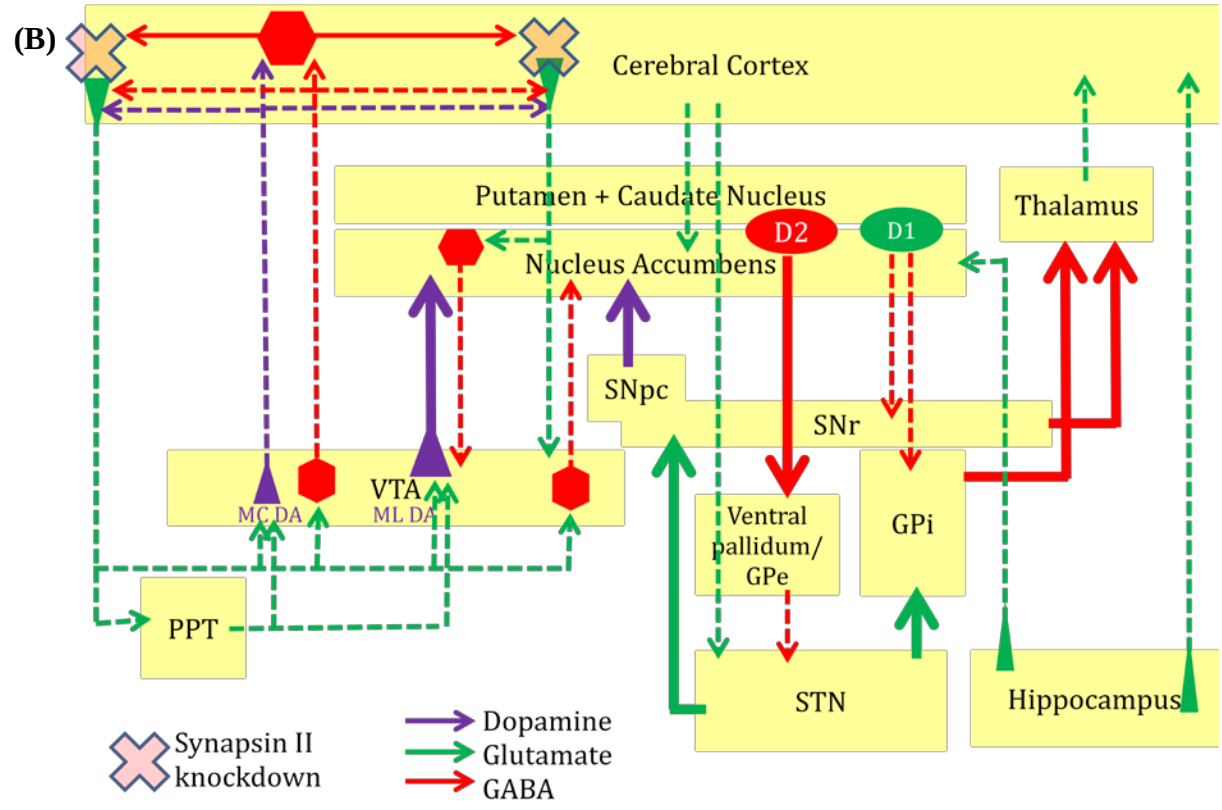

(B)
Cerebral Cortex
Putamen + Caudate Nucleus
Nucleus Accumbens
D2
D1
Thalamus
SNpc
SNr
VTA
MC DA
ML DA
Ventral pallidum/ GPe
GPi
PPT
STN
Hippocampus
Synapsin II knockdown
Dopamine
Glutamate
GABA

Figure 4A-B: (**A**) Diagram indicates oversimplified projections of dopamine, glutamate, and GABA neurocircuitry in a normal brain. Glutamatergic projections from the frontal cortex can affect mesocortical (MC) dopaminergic firing from the VTA back into the frontal cortex and mesolimbic (ML) dopaminergic activity from the VTA to the NAc/striatum (putamen + caudate nucleus). Indirect glutamatergic signalling is also mediated though the pedundulopontine tegmentum (PPT). Stimulation of the GABA neurons in the VTA by cortical glutamatergic projections represents a "braking" system, which modulates dopaminergic activity projecting from the VTA. (**B**) Diagram illustrates proposed dopamine, glutamate, and GABA neurotransmission in a brain inflicted with schizophrenia. A disruption of glutamatergic function is illustrated as the primary site of anomaly which extends to downstream changes in glutamate, dopamine, and GABA signalling within the cortical and subcortical regions of the brain. Deficits in NMDA neurotransmission in the cortex, induced by NMDA antagonists or synaptic alterations (marked with "X") will result in decreased MC dopaminergic activity. The deficits in glutamate transmission from the cortex will also disinhibit the GABA neurons in the NAc and VTA, consequently elevating dopaminergic activity from the VTA projecting into the NAc. Hyperdopaminergic activity within the NAc can result in subsequent decrease of glutamatergic signalling back into the cortex via direct and indirect basal ganglia pathways. Select brain regions implicated in schizophrenia have been illustrated along with the relevant altered projections of the PFC-VTA, PFC-hippocampus, PFC-NAc, and the basal ganglia. Other neuronal targets are likely also involved, but may not be as well understood in the pathology of schizophrenia (Carr et al., 2000). Figures are modified from Carlsson *et al.*, Laruelle *et al.*, and are heavily supplemented with other primary studies and extensive reviews described throughout this thesis (Carlsson et al., 2001; Mueller, Haroutunian, Davis, & Meador-Woodruff, 2004; Laruelle et al., 2003; Carr et al., 2000). GPe – globus pallidus external; GPi – globlus pallidus internal; MC DA – mesocortical dopamine; ML DA – mesolimbic dopamine; PPT– pedunculopontine tegmentum; SNpc – substantia nigra pars compacta; SNr – substantia nigra pars reticulata; STN – subthalamic nucleus; VTA – ventral tegmental area.

1.5. Antipsychotic Drugs

The therapeutic options for schizophrenia are currently limited to the use of typical and atypical antipsychotic drugs. There is no cure for this mental disorder, and prescribed drugs merely manage symptoms of the disease (Roth et al., 2009). Due to disease heterogeneity in schizophrenia, the design of effective therapeutics has remained a challenge to this day. A lack of rationalization of the underlying mechanisms in schizophrenia has hindered drug discovery and drug development has mostly been fortuitous (Tamminga et al., 1997). An unremitting presence of adverse side effects also often results from use of antipsychotic drugs. However, despite the poor treatment adherence and a high rate of treatment-resistant patients, the use of typical and atypical antipsychotic drugs still remain to this day the most effective treatment for schizophrenia.

1.5.1. Typical Antipsychotic Drugs

The first pharmacological treatment, chlorpromazine, was discovered in 1950 to induce a state of calmness and somnolence in surgical patients (Tamminga et al., 2005). In 1952, Delay and Deniker reported chlorpromazine to alleviate psychotic symptoms in patients with schizophrenia, although a known tissue or receptor target was yet to be discovered. Shortly after the introduction of chlorpromazine and haloperidol into the clinic, Parkinson's disease-like EPS became evident with use of these medications. In 1963, Carlsson and Lindquist speculated that antipsychotic drugs blocked monoamine receptors for noradrenaline, dopamine, and serotonin (Tamminga et al., 1997). The earliest outline of the dopamine hypothesis arose in 1967, when Baumeister and Francis

proposed that antipsychotic drugs interfered with dopamine neurotransmission, and that the overstimulation of dopamine receptors may in fact be a part of the etiology of schizophrenia.

Drugs developed thereon were built on the principle of dopamine receptor blockade with the rationale that decreasing dopamine-mediated neurotransmission was therapeutic for psychosis (Tamminga et al., 2005; Tamminga et al., 1997). Like haloperidol and chlorpromazine, other first-generation 'typical' class of antipsychotic drugs that were developed primarily antagonise the dopamine D_2 receptor as its primary mechanism of action (Tamminga et al., 1997; Konradi & Heckers, 2001; Mathews et al., 2007). Positive symptoms have been attributed to an upsurge in dopamine release and an increase in number of high affinity dopamine D_2 receptors (D_2^{High}) in the basal ganglia. As such, dopamine D_2 antagonism with typical antipsychotic drugs allowed for the effective alleviation of positive symptoms but not the negative and cognitive symptoms (Seeman, 2011). Unfortunately, antagonism of >80% of the dopamine D_2 receptors usually result in the production of EPS, which has been consistently associated with the use of typical antipsychotic drugs. EPS can include tardive dyskinesia, bradykinesia, akathisia, dystonia, stiffness, and tremor, amongst others (Lieberman et al., 2001; Tamminga et al., 1997; Mathews et al., 2007). Typical antipsychotic drugs are classified into 2 broad categories, namely the phenothiazines and butyrophenones. Phenothiazines include chlorpromazine, thioridazone, fluphenazine, pericyazine, perphenazine, trifluoperazine, and pipotiazine. Butyrophenones include haloperidol, droperidol, and bromperidol (Mathews et al., 2007).

1.5.2. Atypical Antipsychotic Drugs

Second-generation "atypical" class of antipsychotic drugs came a decade later with the invention of clozapine in 1975. Clozapine was found to be effective in treatment-resistant patients, and efficacious at treating the positive, negative, and cognitive classes of symptoms without inducing EPS (Mathews et al., 2007; Tamminga et al., 1997). Invention of clozapine demonstrated that the therapeutic ability of an antipsychotic drug could be distinguished from its adverse side effects, renewing hope in afflicted patients (Meyer et al., 1997). Unfortunately, use of clozapine was found to cause agranulocytosis and was subsequently withdrawn from the market. More than 20 years later, olanzapine was synthesized by Eli Lilly based on the chemical structure of clozapine, and was found to cause comparatively fewer side effects while still allowing the effective treatment of positive and negative symptoms. Other atypical antipsychotic drugs include risperidone, quetiapine, ziprasidone, aripiprazole, paliperidone, amisulpride, and sertindole (Mathews et al., 2007; Konradi et al., 2001).

Atypical antipsychotic drugs have a greater degree of heterogeneity in their pharmacological mechanism of action. This newer class of drugs differ from typical antipsychotic drugs by having a lower affinity for the dopamine D_2 receptor and a greater affinity for a range of other receptors, including the muscarine, histamine, adrenergic, and serotonin receptors (Mathews et al., 2007; Keefe, Silva, Perkins, & Lieberman, 1999; Lieberman et al., 2005). The most effective therapeutic options for negative and neurocognitive impairments is thought to result from a combined dopamine D_2 and serotonin 5-HT_{2A} receptor antagonism, which is most effectively achieved with the use of

atypical antipsychotic drugs (Horacek et al., 2006; Owen, O'Donovan, & Harrison, 2005). EPS are less common with atypical antipsychotic drug use, which could be attributed to the blockade of serotonin 5-HT_{2A} receptors (Kantrowitz & Citrome, 2008). Due to their more complicated mode of action, atypical antipsychotic drugs have more widespread and temperate effects across various brain regions and neurotransmitter systems. Atypical neuroleptics affect the striatum and the dopaminergic system to a lesser extent while having a more pronounced effect on a range of glutamatergic, serotonergic, and adrenergic systems in a variety of brain regions including the PFC (Konradi et al., 2001). Despite promising efficacy and a decreased likelihood of movement disorders, use of atypical antipsychotic drugs come with their own profile of side effects, including metabolic complications such as weight gain, insulin resistance, hyperlipidemia, and an increased risk towards developing type 2 diabetes mellitus (Mathews et al., 2007).

Long-term studies into the effectiveness of the drugs, such as the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) and the European First-Episode Schizophrenia Trial (EUFEST), concluded that both typical and atypical classes of antipsychotic drugs cause discontinuation in patients (Kantrowitz et al., 2008). The most commonly prescribed atypical antipsychotic drug, olanzapine, however, has been reported to have a lower rate of discontinuation than the most commonly prescribed typical antipsychotic drug, sulpiride (Kantrowitz et al., 2008).

1.5.2.1. Olanzapine

Olanzapine is a thienobenzodiazepine antipsychotic, sold under the brand name Zyprexa® (Eli Lilly and Co., Indianapolis, IN, USA) (Kantrowitz et al., 2008; Tamminga et al., 1997). This second-generation antipsychotic drug was first approved in 1996 by the FDA for the treatment of patients 18 years of age and older (Kantrowitz et al., 2008). Today, olanzapine is a common treatment for patients with schizophrenia and/or bipolar mania (Kantrowitz et al., 2008). Like all other antipsychotic drugs, olanzapine also shares a common blockade of the dopamine D_2 receptor, albeit to a lesser extent than the first-generation typical antipsychotic drugs. Olanzapine possesses a much wider binding profile, and antagonizes the dopamine D_1, D_3, D_4, histamine H_1, muscarinic $M_{1\text{-}5}$, α_1 adrenergic, and serotonergic $5HT_{2A/2C}$, $5HT_3$, $5HT_6$ receptors (Kantrowitz et al., 2008). Olanzapine also binds weakly to $GABA_A$, benzodiazepine, and β-adrenoceptors (Kantrowitz et al., 2008; McCormack & Wiseman, 2004; Tamminga et al., 1997).

In vivo imaging studies demonstrate that olanzapine possesses a relatively low dopamine D_2 receptor occupancy in the striatum (69%) and a higher serotonin 5-HT_2 occupancy in the cortex (84%) (Tamminga et al., 1997). In pharmacokinetic studies, the measured time (T_{max}) for orally administered olanzapine to reach the peak plasma concentration within the body post-administration is 5-6 h, and elimination half-life of the drug is 30-33 h (Kantrowitz et al., 2008; McCormack et al., 2004; Tamminga et al., 1997). Intramuscular administration of olanzapine produces a lower T_{max} of 45 min, with approximately 5 times higher peak concentration and a similar plasma concentration area under the curve than an equivalent oral dose. Regardless of the method of administration,

only approximately 60% of administered olanzapine reaches the systemic circulation with the rest eliminated extensively by first-pass mechanism (Kantrowitz et al., 2008). Oxidation of olanzapine is induced by cytochromes 1A2 and 2D6, with cytochrome 1A2 having the most oxidative effect of these P450 enzymes (Kantrowitz et al., 2008).

The binding profile of olanzapine can be used to explain both the therapeutic and adverse side effects. Preference for the therapeutic as opposed to adverse Parkinson's disease-like effects can seen anatomically through the depolarization in the A10 mesolimbic projection and not the A9 nigrostriatal projection, as well as the restricted *Fos* protein activation in the ventral striatum and medial PFC (mPFC) without extending into the dorsal striatum (Mathews et al., 2007; Tamminga et al., 1997). Blockade of the α_1-adrenoreceptors can lead to orthostatic hypotension, bradycardia, and tachycardia (Kantrowitz et al., 2008; Mathews et al., 2007). Blockade of the muscarinic receptors can lead to anti-cholinergic effects on the parasympathetic system responsible for the involuntary movement of smooth muscles, thus leading to constipation, urinary hesitancy, and dry mouth (Kantrowitz et al., 2008; Mathews et al., 2007). 5-HT_{2C} receptor antagonism can induce weight gain and antagonism of the H_1 receptors can induce somnolence and weight gain (Kantrowitz et al., 2008; Mathews et al., 2007).

When compared to typical antipsychotic drugs, olanzapine displays equivalent efficacy in treating the positive symptoms but superior efficacy at treating the negative symptoms of schizophrenia (Tamminga et al., 1997). Furthermore, olanzapine is least likely amongst the class of atypical antipsychotic drugs to induce EPS, while only causing mild and transient elevations in prolactin at the lower, recommended, dosages

(Kantrowitz et al., 2008). Despite its association with weight gain, diabetes mellitus, and dyslipidemia, robust clinical trials CATIE and EUFEST have demonstrated compelling evidence for its therapeutic value, and the benefits of olanzapine use outweighs the risks in patients with a low baseline risk for metabolic syndrome. With careful monitoring of weight and other metabolic parameters, olanzapine remains the first-line therapy for schizophrenia and an important part of the armamentarium to treat psychotic disorders (Kantrowitz et al., 2008).

1.6. Synapsins

1.6.1. Synapsin Function

Synapsins are the first synaptic vesicle-associated family of proteins to be discovered (Südhof, 2004). This highly conserved family is the most abundant of neuron-specific phosphoproteins, consisting 9% of the total amount of all vesicle proteins (Südhof et al., 1989; Rosahl et al., 1995; Greengard et al., 1993). These presynaptic proteins are important for synaptogenesis, synapse function, synapse maintenance and synaptic plasticity (Greengard et al., 1993; Kao et al., 1999; Bogen et al., 2006). Specifically, synapsins have a crucial role in neurotransmission by regulating the trafficking of synaptic vesicles (Hilfiker et al., 1999). Through dynamic cellular interactions, synapsins selectively and reversibly bind to synaptic vesicles and interact with actin filaments to tether these vesicles to the cytoskeleton of the reserve pool (Greengard et al., 1993). During the firing of an action potential, the influx of Ca^{2+} allows for subsequent phosphorylation of the associated synaptic proteins. Upon

phosphorylation of synapsin, the actin filaments dissociate and liberate synaptic vesicles from the cytoskeleton. This transitions the synaptic vesicles from the reserve to the active pools, thereby regulating the availability of synaptic vesicles ready for exocytosis and successive neurotransmitter release (Greengard et al., 1993; Chen et al., 2004a). Phosphorylation can regulate the availability of synaptic vesicles and alter neurotransmitter release at the synapse. The phosphorylation state of synapsins are increased under the promotion of Ca^{2+}-dependent neurotransmitter release (Greengard et al., 1993). Contrarily, dephosphorylation of synapsins can act as an inhibitory constant preventing the release of synaptic vesicles, of which constraint can be alleviated by phosphorylation with the respective kinases (Hilfiker et al., 1999; Fornasiero, Bonanomi, Benfenati, & Valtorta, 2010; Greengard et al., 1993; Chen et al., 2004a). This process is conceivable to have its effects in learning and memory. A disruption in active synapsin expression levels in the PFC can dysregulate neurotransmission and have detrimental effects on learning; this lends to the manifestation of cognitive deficits and other associated abnormalities often seen in patients with schizophrenia (Greengard et al., 1993).

Synapsins are important for short-term synaptic plasticity, axonal outgrowth and synaptogenesis (Rosahl et al., 1995; Südhof, 2004). Synapsin knockout mice (I, II, or both) exhibit abnormalities in neurotransmission, reduced synapse numbers, and a lack of vesicle clusters at presynaptic sites (Kao et al., 1999; Greengard et al., 1993). Although both synapsins I and II serve to maintain synaptic vesicle numbers at nerve terminals, they individually play distinct roles in the development of cultured hippocampal neurons;

deletion of synapsin II alone has a greater effect on lammelipodial formation, neurite formation, and axon differentiation, whereas deletion of synapsin I alone more prominantly affects synapse formation (Ferreira et al., 1998). Transfection of synapsin IIb cDNA in a neuroblastoma-glioma cell line results in the formation of increased nerve terminals and synaptic vesicles within each terminal (Kao et al., 1999; Greengard et al., 1993). Synapsin IIb cDNA transfection also increases the expression levels of other associated synaptic vesicle proteins within the nerve terminal, thus accelerating the development and maturation of neurons (Kao et al., 1999; Greengard et al., 1993). An inhibition of synapsin II expression by antisense oligonucleotides (AS) in cultured hippocampal neurons, on the other hand, was found to inhibit axon elongation, and interfere with the formation and maintenance of synapses (Kao et al., 1999; Ferreira et al., 1995; Ferreira et al., 1994). A synapsin II knockout animal was phenotypically more severe than the synapsin I knockout animal, with a double knockout of I and II causing the most drastic changes including a 50% reduction in synaptic vesicle numbers (Rosahl et al., 1995; Südhof, 2004). Synapsin III knockout mice exhibited the least drastic of changes, such as a 5% increase in synaptic vesicle density and altered GABA signalling (Südhof, 2004).

1.6.2. Synaptic Vesicle Cycle

Neuronal signalling is mediated by the trafficking cycle of the synaptic vesicle and neurotransmitter release (Südhof, 2004). This process is tightly regulated by intracellular messengers and extracellular modulators (Südhof, 2004). Neurotransmitters are actively

taken up into the synaptic vesicles, which then cluster at the active zone/readily releasable pool of the synapse. These vesicles are primed into a state of competence to become Ca^{2+} responsive (Schweizer & Ryan, 2006). The extent of the 'Soluble NSF Attachment Protein Receptor, or 'SNARE', complex formation and/or the distance to the next Ca^{2+} entry site determines the degree by which a vesicle is primed and whether or not the docked vesicle will exocytose (Schweizer et al., 2006). During an action potential, voltage-gated Ca^{2+} channels open, causing an influx of Ca^{2+}, which then triggers the pore opening of release-ready vesicles and the exocytosis of the contained neurotransmitters (Südhof, 2004; Schweizer et al., 2006). Following fusion pore opening, synaptic vesicles then endocytose following three alternate pathways: (1) Vesicles can be reacidified and refilled with neurotransmitters after a closure of the fusion pore while remaining docked at the readily releasable pool of the plasma membrane in a process called "kiss-and-stay", (2) vesicles can undock and endocytose from the readily releasable pool to mix with vesicles at the reserve pool, reacidify and refill with neurotransmitters locally in a process called "kiss-and-run", or (3) vesicles can also endocytose through a slower third process via clathrin-coated pits, reacidify and refill with neurotransmitters directly or after passing through an endosomal intermediate (Südhof, 2004; Atluri & Ryan, 2006). The involvement of the endosomal intermediate in clathrin-dependent endocytosis is currently under debate, although evidence has been provided for the physiological importance of the endosomal pathway for synaptic vesicle recycling under some conditions (Südhof, 2004). The two faster processes of recycling may utilize transient fusion pores at a lower stimulation frequency, whereas the slower process of recycling via clathrin-coated pits may undergo a

full collapse of the vesicle into the plasma membrane at higher stimulation frequencies (Südhof, 2004). The selection between the types of endocytotic pathways is currently not known, although some studies suggest the differing capacities of the vesicle could result from the maintenance of different frequency stimulations (Südhof, 2004). Steady-state fast local recycling at low frequencies is easier to maintain than state-state release at high frequencies. Moreover, the endosomal-mediated recycling pathway makes up for its lack of speed with its comparatively larger vesicular capacity (Südhof, 2004). Another hypothesis suggests that the endosomes could serve as a sorting station to separate the "defective" and "healthy" vesicles when the synapse undergoes periods of stress (Südhof, 2004).

Synaptic vesicles contain two classes of obligatory components, namely the vesicular transport proteins and trafficking proteins (Südhof, 2004). Vesicular transport proteins are involved in neurotransmitter uptake, and include both the vacuolar-type protein pump, which acidifies the vesicle and generates the electrochemical gradient to fuel the uptake of neurotransmitters through ATPase activity, as well as neurotransmitter transporters, including VGLUTS 1-3, VGAT, and VMAT-2, which mediate the uptake of their respective neurotransmitters into the synaptic vesicle (Südhof, 2004). Trafficking proteins participate in vesicle exocytosis, endocytosis, and synaptic vesicle recycling. Examples of trafficking proteins include SNARE proteins such as synaptobrevin, synaptaxin 1, and SNAP-25 (Südhof, 2004). Additionally, synapsins are also expressed in abundance on the surface of synaptic vesicles. Synapsins may function to anchor synaptic

vesicles through the binding of a meshwork of filaments and cytoskeletal elements, such as actin (**Figure 5**) (Südhof, 2004; Schweizer et al., 2006).

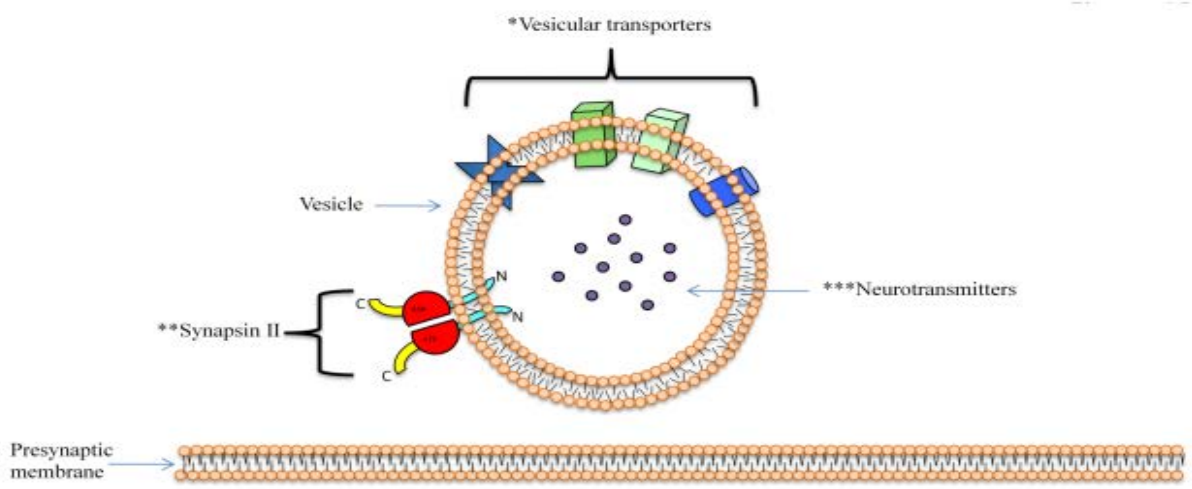


Figure 5: Oversimplified illustration of the interaction of synapsin II with the associated synaptic vesicle. At the presynaptic cleft, synapsins help tether synaptic vesicles to the reserve pool. In addition, specified vesicular transporters also associate with synaptic vesicles to help load their respective neurotransmitters into the vesicles at presynaptic terminals (Cesca, Baldelli, Valtorta, & Benfenati, 2010; Gitler, Cheng, Greengard, & Augustine, 2008). *Select examples of vesicular transporters include VGLUT-1, VGULT-2, VGAT, and VMAT-2. ** Various other synapsin isoforms and synaptic proteins can interact with and tether to the synaptic vesicle depending on the brain region and neuron of interest. ***Select examples of neurotransmitters contained in synaptic vesicles include glutamate, dopamine, and GABA.

1.6.3. Synapsin Structure and Subtypes

Synapsins are coded by 3 genes: SYNI, SYNII, and SYNIII, which are located on chromosome X, 3 and 22, respectively (Hilfiker et al., 1999). Alternate splicing of each synapsin gene gives rise to a total of 10 synapsin isoforms (**Figure 6**) (Hilfiker et al., 1999; Dyck & Mishra, 2012a; Cesca et al., 2010; Dyck et al., 2012b) These proteins are found in virtually all presynaptic terminals, but the specific isoform of synapsin expressed may be dependent on the neuron type (Bogen et al., 2006; Greengard et al., 1993). Although its importance in neuronal functioning is established, there is still much unknown about the specific functions of synapsin within the neuron (Südhof, 2004). Synapsins I and II are found in mature synapses, while synapsin III is developmentally controlled and is expressed in lesser abundance (Südhof, 2004; Greengard et al., 1993; Hilfiker et al., 1999).

The NH_2-terminus and central domains of all synapsin isoforms are highly conserved and primary structural differences amongst the various isoforms are restricted to the COOH-terminals of the molecule (Hilfiker et al., 1999; Südhof, 2004). The short N-terminal domain is approximately 20 residues (Südhof, 2004). Synapsins bind the lipid surfaces of vesicles via the N-terminal while the elongated, basic, and hydrophilic C-terminal of synapsin binds to the protein component of vesicles; this binding is diminished upon phosphorylation of synapsins by a range of kinases at the serine residue at domain A (Südhof, 2004; Pieribone et al., 1995; Kao et al., 1999; Greengard et al., 1993; Hilfiker et al., 1998). Domain A contains phosphorylation sites for cyclic adenosine 3',5'-monophoshate (cAMP)-dependent protein kinase A (PKA),

Ca^{2+}/calmodulin-dependent protein kinase I (CaMKI), and CaMKIV, which are conserved phosphorylation sites for all synapsin isoforms (Fornasiero et al., 2010). The C domain is approximately 300 residues and accounts for more than half of the sequence length in most synapsin isoforms (Südhof, 2004). A conserved tyrosine site is found in domain C for phosphorylation by tyrosine kinase *Src* in synapsins I and II (Fornasiero et al., 2010). Domain C is the most conserved amongst invertebrates, and has been suggested to be essential for binding of the lipid bilayer of synaptic vesicles to actin filaments in the cytoskeleton (Südhof, 2004; Pieribone et al., 1995; Kao et al., 1999; Greengard et al., 1993; Hilfiker et al., 1998). The central C domain forms a constitutive dimer and binds ATP with high affinity (Südhof, 2004; Fornasiero et al., 2010; Pieribone et al., 1995; Kao et al., 1999; Greengard et al., 1993; Hilfiker et al., 1998). This ATP-binding property behaves differently amongst the different synapsins; synapsin I only binds ATP in the presence of Ca^{2+}, synapsin II binds ATP irrespective of Ca^{2+}, and synapsin III binds ATP only in the absence of Ca^{2+} (Südhof, 2004; Hosaka & Südhof, 1998). The D-domain contains proline-rich strand binding SH3 domains, as well as phosphorylation sites for CAMKII, mitogen-activated protein kinase (MAPK) and cyclin-dependent kinase 1/5 (Cdk 1/5) (Südhof, 2004; Fornasiero et al., 2010; Schweizer et al., 2006; Skoblenick et al., 2010). Only one isoform for synapsin III is indicated in **Figure 6**, but multiple synapsin III products have been found in the adult brain (Fornasiero et al., 2010). A range phosphorylation sites exist on the various synapsin isoforms, and serve to differentially regulate the binding ability of synapsins to synaptic vesicles, cytoskeletal

elements, as well as determine the function of synapsins within developing neurons and mature terminals (**Figure 6**) (Hosaka et al., 1998; Fornasiero et al., 2010).

Domains A-C are shared amongst all isoforms of synapsins. Domain E is shared amongst all the “a” isoforms and is thought to have a specific role in the clustering of synaptic vesicles and the maintenance of the reserve pool through actin interactions (Südhof, 2004; Fornasiero et al., 2010; Gitler et al., 2008; Pieribone et al., 1995; Kao et al., 1999; Greengard et al., 1993; Hilfiker et al., 1998). Moreover, domain E may be involved in the forming of synapsin dimers; the "a" isoforms may dimerize to bring weaker targeting isoforms, such as Ib, to synaptic terminals (Fornasiero et al., 2010; Monaldi et al., 2010). Domains B, D, and F-J are poorly conserved amongst isoforms and are specific to each synapsin gene (**Figure 6**) (Südhof, 2004; Pieribone et al., 1995; Kao et al., 1999; Greengard et al., 1993; Hilfiker et al., 1998).

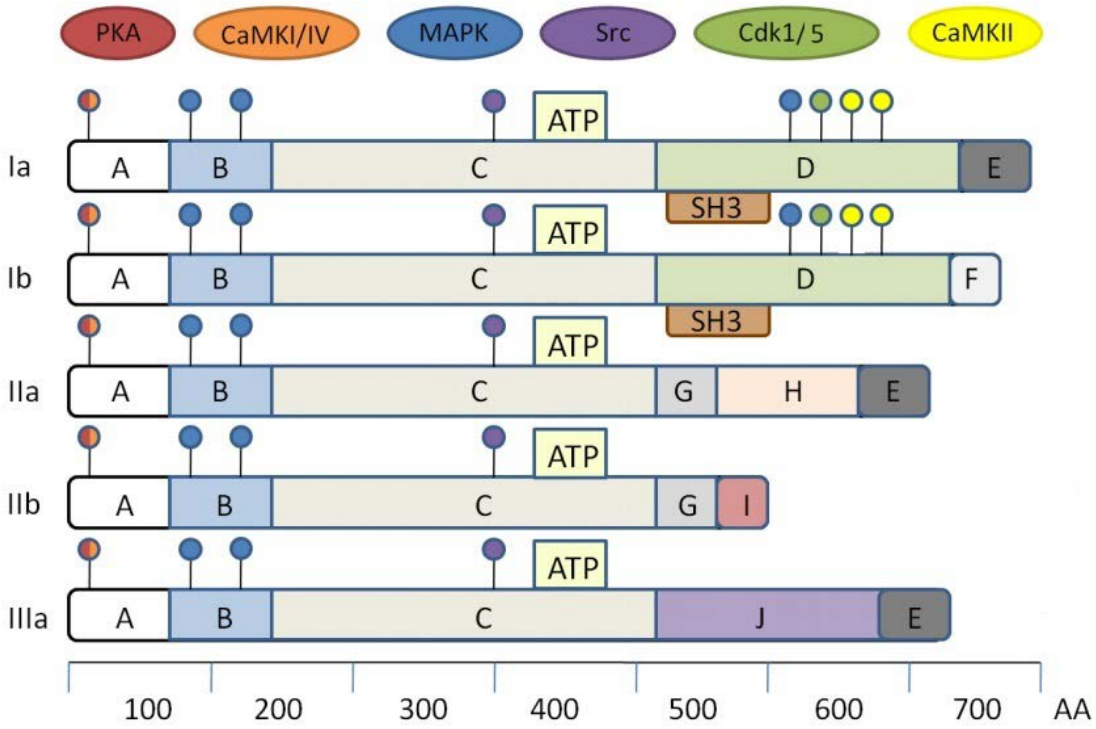


Figure 6: The various domains and isoforms of the mammalian synapsin family, as illustrated by Dyck *et al.* (Südhof, 2004; Hilfiker et al., 1999; Monaldi et al., 2010; Fornasiero et al., 2010; Dyck et al., 2012a; Pieribone et al., 1995; Kao et al., 1999; Greengard et al., 1993; Hilfiker et al., 1998). The length of the polypeptide chains is shown in number of amino acid residues at the bottom of the figure. Phosphorylation sites of the respective kinases are colour-coded, and indicated at positions Ser^{9}, Ser^{62}, Ser^{67}, Ser^{549}, Ser^{551}, Ser^{566}, and Ser^{603} (Monaldi et al., 2010; Fornasiero et al., 2010).

1.6.4. Synapsin Gene Regulation

Through DNA-protein binding assays, binding sites for transcription factors inducible zinc-finger transcription factor, early growth response factor (EGR-1), polyoma enhancer activator 3 (PEA-3), and activating protein 2-alpha (AP-2α) have been found within the 5'-flanking region of the synapsin II gene (Petersohn, Schoch, Brinkmann, & Thiel, 1995). The 5' flanking region of the synapsin II gene also contains numerous Sp1 sites, and lacks TATA and CAAT boxes. Transcriptional start sites and proximal promotor regions of the synapsin II gene was found to contain GC-rich sequences, which suggest methylation having an effect on synapsin II expression and regulation (Petersohn et al., 1995).

Despite the high degree of homology between synapsin I and II genes, promotors for both these genes reveal low homology, and suggest the involvement of differing transcription factors (Petersohn et al., 1995). Contrary to the synapsin II gene, synapsin I does not contain a promotor region for AP-2α (**Figure 7**) (Skoblenick et al., 2010). The synapsin I gene also contains two additional EGR-1 binding sites, a neural-restrictive silencer element next to one EGR-1 binding region and a cAMP-response element next to another (**Figure 7**) (Skoblenick et al., 2010). EGR-1 is termed a cellular immediate early gene and the EGR-1 gene expression is highly responsive to neuronal stimulation (Petersohn et al., 1995). Studies have shown that an induction of LTP causes an increase in EGR-1 mRNA and protein, and subsequent activation of the synapsin I gene expression 3 hours later. Given the presence EGR-1 on the synapsin II gene, a similar function has been hypothesized for synapsin II following the induction of LTP

(Petersohn et al., 1995). The PEA-3 promotor is influenced by serum promotors, tumor promotors, and the gene products of several non-nuclear oncogenes including v-raf, v-src, Ha-ras, and polyoma middle T-antigen (Petersohn et al., 1995). These proteins are involved in the signal transduction cascade activating the MAP kinase (Petersohn et al., 1995). When activated, MAP kinase translocates into the nucleus and activates the transcription of transcription factors including PEA-3. Petersohn *et al.* provides evidence for extracellular signal molecules activating synapsin II expression through the MAP kinase pathway and the PEA-3 promotor (Petersohn et al., 1995). The two transcription sites on the synapsin II gene for AP-2 is located in close proximity, making it unlikely that both sites would be occupied at the same time (Petersohn et al., 1995). Upon transcription, post-translational modifications of AP-2, and not increase in AP-2 mRNA expression levels, mediate the effects of protein kinase C and cAMP (Petersohn et al., 1995). A study from our laboratory indicated that only AP-2α, and not EGR-1 nor PEA-3, was found to have a role in the dopamine-mediated regulation and synthesis of synapsin II (**Figure 7**) (Skoblenick et al., 2010). Dopamine stimulation causes an increase in intracellular cAMP levels, which then activates protein kinase A (PKA) (Skoblenick et al., 2010). PKA may proceed to activate AP-2α and initiate synapsin II production (**Figure 7**) (Skoblenick et al., 2010).

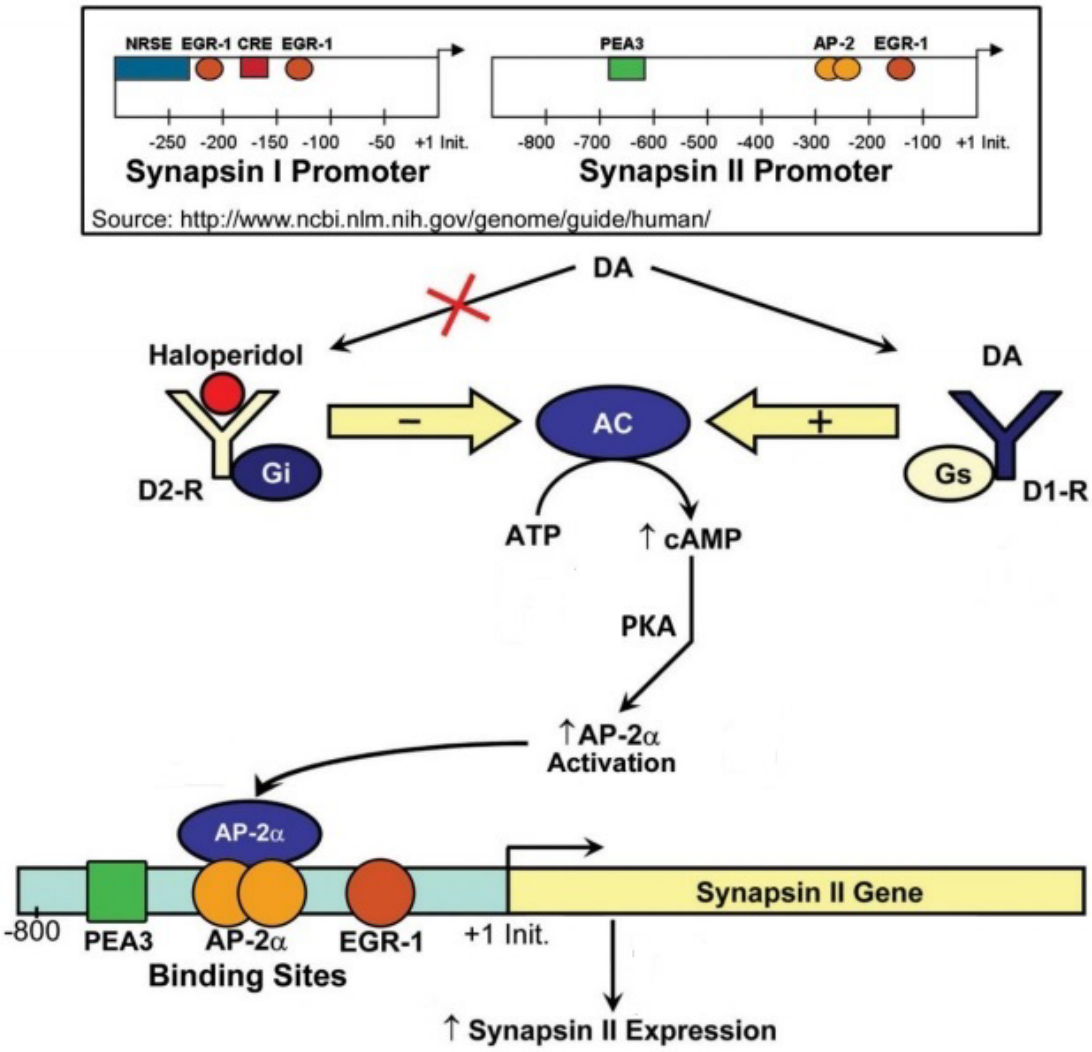


Figure 7: Hypothesized mechanistic regulation of synapsin II expression levels by dopamine receptors, as postulated by Skoblenick *et al.* (Skoblenick et al., 2010). D_2 receptor antagonist or D_1 receptor agonist binding causes the subsequent increase of intracellular cAMP levels, which then activate PKA and AP-2α. Activated AP-2α then binds to the synapsin II promotor and initiates synapsin II synthesis. Transcription factors PEA-3 and EGR-1 were not found to be involved in the regulation of synapsin II.

1.6.5. Synapsin Associations with Specific Neurotransmitters

All synapses contain different complements of synapsin isoforms. By controlling the storage and mobilization of synaptic vesicles, synapsins are important regulators of synaptic vesicle dynamics (Gitler et al., 2008). Synapsins have unique functions at excitatory and inhibitory synapses; the presence or absence of synapsins can differentially regulate neuron signalling, neuron depression kinetics, and basal amounts of neurotransmitter release at glutamatergic, GABAergic, and dopaminergic neurons (Gitler et al., 2008; Gitler et al., 2004). A deletion in each of the three synapsin genes produces different phenotypes, indicating separate functions for each of the various synapsin isoforms. Gitler *et al.* indicated that synapsins function to maintain the size of the reserve pool within excitatory neurons, a function of which is primarily mediated by synapsin II. Moreover synapsins also function to maintain both the size of the readily releasable pool and the reserve pool at inhibitory neurons (Gitler et al., 2004). Differences in modulation suggest differing mechanisms responsible for the distribution of synaptic vesicles to the readily releasable pool and the reserve pool within different classes of neurons (Gitler et al., 2004).

In a synapsin triple knockout (i.e. I, II, and III) mouse, only the synapsin IIa isoform served the unique role of rescuing the deficits in synaptic vesicle density and maintaining the increased number of vesicles within the reserve pool of glutamatergic synapses (Gitler et al., 2008). Furthermore, only the synapsin IIa isoform was capable of rescuing the defects in synaptic depression kinetics and regulate synaptic vesicle mobilization at glutamatergic synapses (Gitler et al., 2008). Synapsin IIb may have a

similar tendency, but its rescuing abilities did not reach statistical significance. The other isoforms of synapsin Ia, Ib, or IIIa did not have the same rescuing effects (Gitler et al., 2008). Studies indicate that synapsin IIa is the main modulator of synaptic vesicle pools and signalling at glutamatergic neurons (Gitler et al., 2008). Synapsin I isoforms may have a more central and profound role within inhibitory interneurons since the genetic ablation of synapsin I was found to interfere with GABA release through a decrease in the readily releasable pool size, as well as a reduction in the recycling and refilling rate of synaptic vesicles within the reserve pool of inhibitory neurons (Baldelli, Fassio, Valtorta, & Benfenati, 2007). On the contrary, triple knockout cells were found to have an enhancement in the quanta of catecholamine released, of which phenotype was rescued by synapsin IIa (Villanueva, Thornley, Augustine, & Wightman, 2006). Evidence indicates that synapsin IIa is not required for, but may rather serve as a negative modulator of, catecholamine release (Villanueva et al., 2006). Functions of synapsins differ for vesicles containing different types of neurotransmitters; the absence of synapsins results in a decrease in glutamate and GABA transmission, while causing an increase in catecholamine release (Villanueva et al., 2006).

1.6.6. Synapsin II and Schizophrenia

Several candidate genes have been found to be responsible for the etiology of schizophrenia, many of which include those central to synaptic function, such as the synapsin family of proteins (Owen et al., 2005; Harrison & Owen, 2003). Synapsin II is located on chromosome 3p25, which is a region suggested to have significant positive

genetic linkage towards the development of schizophrenia (Lewis et al., 2003). As such, synapsin II is a candidate gene for increased susceptibility and may be involved in the etiology of this disorder (Chen et al., 2004a; Saviouk, Moreau, Tereshchenko, & Brzustowicz, 2007; Chen et al., 2004b; Dyck et al., 2009; Dyck et al., 2007; Dyck et al., 2011). Synapsin II mRNA and protein levels have been found to be significantly reduced in the brains of patients with schizophrenia when compared to normal subjects (Chen et al., 2004a; Mirnics, Middleton, Marquez, Lewis, & Levitt, 2000; Vawter et al., 2002; Guest, Dyck, Shethwala, & Mishra, 2010; Chen et al., 2004b). Furthermore, treatment with antipsychotic drugs, such as haloperidol and olanzapine, has been found to increase expression levels of synapsin II in the rat and human brain (Guest et al., 2010; Chong, Skoblenick, Morin, Xu, & Mishra, 2006; Dyck et al., 2011). Synapsin II has also been previously shown to be directly regulated by dopamine receptors; treatment with D_1 receptor agonists (ie. SKF 38393) and D_2 receptor antagonists (ie. haloperidol) increased expression levels of synapsin II, while treatment with D_1 receptor antagonists (ie. SCH 23390) decreased the expression levels of synapsin II both *in vitro* (primary cells) and *in vivo* (rats) (**Figure 7**) (Chong, Young, & Mishra, 2002; Chong et al., 2006). Evidence substantiates a crucial role for synapsin II in the pathophysiology and therapeutic mechanisms for the treatment of schizophrenia.

Due to its critical influence on neurotransmitter regulation and synaptic maintenance, a disruption in the expression levels of synapsin II in the PFC may lead to a dysregulation in presynaptic function, an imbalance in brain circuitry, and subsequently promote the onset of mental illnesses such as schizophrenia (**Figure 4**) (Chen et al.,

2004a; Mirnics et al., 2001; Mirnics et al., 2000; Owen, 2005; Chen et al., 2004b). Much of what we already know about synapsin II and its role in schizophrenia has been derived from animal knockout and knockdown studies (Rosahl et al., 1995; Dyck et al., 2009; Dyck et al., 2011; Dyck et al., 2007). Our laboratory has previously reported that synapsin II knockout mice bear behavioural abnormalities similar to those of animal models of schizophrenia, such as locomotor hyperactivity, social withdrawal, and deficits in prepulse inhibition (Dyck et al., 2009; Dyck et al., 2011; Dyck et al., 2007). Moreover, synapsin II knockdown in specifically the mPFC of an adult rat was found to cause similar schizophrenia-like deficits, including deficits in prepulse inhibition, locomotor hyperactivity, social withdrawal, hypofunction in the PFC (i.e. hypofrontality) (not published), deficits in the 8-arm radial maze, and poor performance in the 5-choice serial reaction time task (not published) (Dyck et al., 2009; Dyck et al., 2011; Dyck et al., 2007). These symptoms are all reminiscent of the positive, negative, and cognitive symptom domains of schizophrenia. Immunoblotting also revealed additional neurochemical changes within the mPFC of the synapsin II knockdown animal, including a reduction in glutamate vesicular transporters (i.e. VGLUT-1, VGLUT-2), vesicular GABA transporter (i.e. VGAT), and GABA synthesizing enzyme $GAD_{65/67}$ (not published) (Dyck et al., 2011). There were no changes in protein expression levels of the vesicular monoamine transporter 2 (i.e. VMAT-2) and the dopamine active transporter (DAT) in the mPFC (not published) (Dyck et al., 2011). Neurochemical results indicate a reduction in glutamate and GABA signalling, an unaltered dopamine signalling, within the mPFC resulting from synapsin II knockdown. All abnormalities in the measured

parameters of the adult synapsin II model were reversed with administration of antipsychotic drug, olanzapine (Dyck et al., 2011). Interestingly, a synaptic-neurodevelopmental model of schizophrenia has been proposed by Mirnics *et al*., whereby genetic predisposition to defected synaptic protein expression and altered signalling during development, along with possible inadequate adaptation to compensate for deficits during early childhood and/or adolescence, can lead to the manifestation of schizophrenia in the developmental time course (Laruelle et al., 2003; Chen et al., 2004b; Mirnics et al., 2001). These faults in presynaptic gene expression and deficits in synaptic functions may lead to PFC dysfunction and the ensuing cognitive deficits, along with the positive and negative symptoms commonly seen in patients with schizophrenia (Laruelle et al., 2003; Mirnics et al., 2001). Accordingly, the current thesis explores the neurodevelopmental role of synapsin II in the synthesis of abnormalities pertinent to schizophrenia.

1.7. Modeling Schizophrenia

Over fifty animal models of schizophrenia have been described over the past 30 years (Tseng et al., 2009). Each model presents its own set of advantages and limitations, as well as differ in the degree to which they model the various focal aspects of the disease (Tseng et al., 2009). The use of animal models has been essential towards our understanding of schizophrenia, the development of symptoms, the plausibility of theories, and the underlying mechanisms involved in the disease (Lipska, 2004).

Moreover, animal models have significantly contributed to the design and development of current and new therapies used to treat schizophrenia (Lipska, 2004).

1.7.1. Genetic models

Despite the fact that schizophrenia is mental disorder with prominent genetic heritability of up to 80%, there is no single gene found to be causal and sufficient to explain this complex heterogeneous disorder (Jones, Watson, & Fone, 2011). A majority of genes found implicated in this disease are involved in neuroplasticity, synaptogenesis, and neurotransmission (Jones et al., 2011). Genes of susceptibility most likely act in a synergistic manner in combination with epigenetic processes and early-life events to determine the outcome of disease (Jones et al., 2011). Researchers have strove to develop endophenotype models, which will present stable and measurable features to distinguish between overt manifestations as opposed to risk genes for schizophrenia (Jones et al., 2011). Rare genetic mutations have conferred a high enough risk to penetrate disease development, and only a selection of genetic models will be briefly reviewed in this section (Jones et al., 2011).

The Disrupted- In- Schizophrenia 1 (DISC-1) gene was one of the earliest genes found implicated in schizophrenia (Jones et al., 2011). DISC-1 is a synaptic gene expressed early on in development and is involved in prenatal and postnatal neuronal development (Jones et al., 2011). DISC-1 is important for periods of synaptogenesis, neuronal migration, and synaptic plasticity (Jones et al., 2011; Pletnikov et al., 2008). Although some studies have found positive linkage between DISC-1 and schizophrenia, other studies have found otherwise. Animals with mutated DISC-1 have enlarged lateral

ventricles, reduced cortical thickness, and reduced brain volume (Jones et al., 2011; Hikida et al., 2007; Pletnikov et al., 2008). Some studies also show a reduction in parvalbumin immunoreactivity within the mPFC and hippocampus, while others do not (Jones et al., 2011; Pletnikov et al., 2008). Deficits in prepulse inhibition were reversed with typical and atypical antipsychotic drug treatment in some DISC-1 transgenic animals but not in others (Jones et al., 2011). Hyperactivity, social interaction, and deficits in working memory and executive function are other behavioural phenotypes found in some DISC-1 animals but not others (Jones et al., 2011; Hikida et al., 2007). This discrepancy in results could likely be attributed to the method of inducing the DISC-1 transgenic animal as opposed to changes in DISC-1 gene expression (Jones et al., 2011). There is, however, still more work to be done to establish this genetic model of schizophrenia.

Dysbindin is a synaptic protein, which regulates exocytosis, vesicle biogenesis, and receptor trafficking during excitatory synaptic transmission (Jones et al., 2011; Numakawa et al., 2004). Found on gene dystobrevin-binding protein 1 (DTNBP1), this gene is found to have the most promise as mutations of this gene show strong correlations with disease susceptibility and onset in patients (Jones et al., 2011; Straub et al., 2002). Dysbindin mutants show hyperactivity, altered working memory, impaired novel object recognition, and reduced social interaction, with inconsistent changes in prepulse inhibition responses (Jones et al., 2011; Feng et al., 2008). Reelin is another gene implicated in synaptic formation and plasticity, of which expression levels have been found to be significantly reduced in the cerebellum, hippocampus, and frontal cortex of patients with schizophrenia (Jones et al., 2011; Fatemi, 2001). Reelin knockout mice

display gross abnormalities far more extreme that what is seen in schizophrenia, with few cognitive deficits, mixed prepulse inhibition responses, and reduced dendritic spine density in the frontal cortex and hippocampus (Jones et al., 2011; Fatemi, 2001). Another candidate gene for schizophrenia is NRG-1 and its predominant receptor partner, ErbB4 (Jones et al., 2011; Neddens & Buonanno, 2010). NRG-1 is a pleitrophic growth factor involved in excitatory and inhibitory transmission in the mature brain, synaptogenesis, neuronal migration, glial formation, myelination, and neuron-glial interaction (Jones et al., 2011; Shamir et al., 2012). Homozygous knockout of NRG-1 is developmentally lethal in mice, but viable heterozygous knockouts have been developed with distinct schizophrenia-like alterations (Jones et al., 2011; Shamir et al., 2012). The variable knockouts include deletion of the epidermal growth factor (EGF)-like domain, deletion of the transmembrane domain of NRG-1, deletions of the immunoglobulin (Ig) domain, and deletion of the receptor ErbB4 (Jones et al., 2011; Shamir et al., 2012; Neddens et al., 2010). Differing knockouts are associated with the production of varying and overlapping schizophrenia-like behavioural and neurobiological alterations (Jones et al., 2011; Shamir et al., 2012). Construct validity of NRG-1-ErbB4 models are, however, questionable since the clinical manifestation of schizophrenia is associated with NRG-1 hyperfunction, and not hypofunction as seen in these transgenic animal models (Jones et al., 2011). Further work is required before the functional relevance of these genetic changes can be evaluated for therapeutic purposes.

1.7.2. Pharmacological models

Amphetamine (1-methyl-2-phenethylamine) is a psychostimulant first invented in 1887, and is commonly used to treat disorders including attention deficit, narcolepsy, and historically, obesity (Fleckenstein, Volz, Riddle, Gibb, & Hanson, 2007). Main targets for this compound include the DAT and the VMAT-2, amongst many others (Fleckenstein et al., 2007). There are several mechanisms by which amphetamine can induce the release of monoamines, and in particular that of dopamine (Fleckenstein et al., 2007). Amphetamine may compete for the same binding site as dopamine, thus partake in DAT-assisted transportation into the cells and increase the probability of dopamine being transported down its concentration gradient into the synapse (Fleckenstein et al., 2007; Calipari & Ferris, 2013). Amphetamine may also diffuse into vesicular membranes, occupy vesicles, and cause the accumulation of cytoplasmic dopamine (Fleckenstein et al., 2007; Calipari et al., 2013). By binding VMAT-2, amphetamine prevents dopamine uptake, and increases cytoplasmic dopamine concentrations, further promoting the exchange of dopamine down its concentration gradient and into the synapse (Fleckenstein et al., 2007; Calipari et al., 2013). Regardless of the receptor target or mechanism, the general consensus is that amphetamine facilitates dopamine release by preventing uptake of dopamine from the synapse, thus allowing for increased dopaminergic signalling (Fleckenstein et al., 2007; Calipari et al., 2013).

The amphetamine-induced sensitization animal model of schizophrenia has been long viewed as a model of psychosis. Amphetamine was first found to induce psychosis in non-psychotic individuals in the 1950s, of which symptoms were described as auditory

hallucinations and persecutory delusions representing that of the positive symptoms of schizophrenia (Featherstone et al., 2007; Peleg-Raibstein et al., 2008). The regime for amphetamine sensitization involves chronic amphetamine administration in intermittent escalating doses, followed by a period of withdrawal (Peleg-Raibstein et al., 2008). Both the dosage of amphetamine and time period of withdrawal varies between studies (Peleg-Raibstein et al., 2008). In rodents, chronic amphetamine administration was found to induce persistent sensitization, as measured by hyperactivity induced by acute amphetamine challenge, deficits in prepulse inhibition, and deficits in PFC-dependent tasks like the attentional set shifting task and the five-choice serial reaction time task (Peleg-Raibstein et al., 2008). The positive symptoms have been easy to replicate in this model of the disease, but the negative and cognitive classes of symptoms have been a challenge to replicate (Peleg-Raibstein et al., 2008; Mouri, Noda, Enomoto, & Nabeshima, 2007). In addition to dendritic morphological changes, amphetamine sensitized animals also show an increase in dopamine efflux from the NAc and dorsal striatum and diminished excitability of prefrontal cortical neurons (Featherstone et al., 2007; Peleg-Raibstein et al., 2008). Since the amphetamine-induced model is based on the manipulation of the dopaminergic system, antipsychotic drugs primarily affecting dopamine receptors are found to be most effective at attenuating symptoms (Peleg-Raibstein et al., 2008). Pre-treatment with antipsychotic drug, haloperidol or clozapine, was found to prevent the effects of amphetamine sensitization (Peleg-Raibstein et al., 2008).

Increasing evidence has been presented over the recent years supporting the role of a hypoglutamatergic system as the primary pathological change in schizophrenia (Jones et al., 2011). Pharmacological evidence for the role of glutamate in schizophrenia comes from findings on the blockade of the NMDA receptor with non-competitive antagonists, like ketamine or PCP. These agents induce hallucinations and delusions, which are commonly seen in patients with schizophrenia, in otherwise healthy human subjects (Jones et al., 2011; Mouri et al., 2007). In healthy subjects, PCP administration resulted not only in psychosis, but also progressive withdrawal, a poverty of speech, and an impairment of cognitive performance, akin to three classes of symptoms in schizophrenia (Jones et al., 2011). In rodents, acute PCP administration causes hyperlocomotion, social withdrawal, deficits in prepulse inhibition, and deficits in cognition (Jones et al., 2011). A limitation to acute PCP treatment is the impaired motor function and motivation (Jones et al., 2011). Chronic treatment of PCP was found more likely to enhance the production of symptoms which persist beyond PCP cessation (Jones et al., 2011; Mouri et al., 2007). With chronic PCP sensitization, animals display an increase in locomotor activity upon PCP challenge and a dose-dependent reduction in social interaction, all of which are attenuated by typical and atypical antipsychotic drugs (Jones et al., 2011; Mouri et al., 2007). The social deficits induced in mice were, however, only attenuated with atypical antipsychotic drug treatment (Jones et al., 2011; Mouri et al., 2007). While some cognitive deficits persist for weeks post-PCP cessation, other cognitive tasks were found to only display cognitive impairments shortly after the dosing regimen (Jones et al., 2011). Typical antipsychotic drugs like haloperidol were

less effective at reducing cognitive deficits as when compared to a number of atypical antipsychotic drugs (Jones et al., 2011). Impairments in prepulse inhibition are, similarly, not sustained with chronic PCP treatment, and deficits diminish within days of PCP cessation (Jones et al., 2011). Moreover, prepulse inhibition deficits in PCP treated animals were only reversed with typical, but not atypical, antipsychotic drug treatment (Mouri et al., 2007).

Neurochemical changes resulting from chronic PCP treatment include a hyper-responsive mesolimbic dopamine system to stress and amphetamine challenge, as well as a reduction in dopamine concentration levels in the PFC (Jones et al., 2011). PCP-treated rats also display cortical glutamatergic hypofunction, as evidenced with a reduction in glutamate concentration levels and an increase in expression levels of glutamate-aspartate transporter levels in PFC (Jones et al., 2011; Mouri et al., 2007). As well, chronically treated rats display hypometabolism with the display of reduced basal glucose utilization in the PFC of the brain (Jones et al., 2011; Mouri et al., 2007). Similar to chronic PCP treatment, rats treated chronically with dizocilpine (MK-801), an uncompetitive antagonist of the NMDA receptor, show a reduction in the number of parvalbumin-containing neurons in the dentate gyrus and CA1 regions of the hippocampus (Jones et al., 2011; Mouri et al., 2007). However, chronic PCP treatment also cause changes within the PFC which are not found with MK-801 treatment, such as a reduction of the parvalbumin-immunoreactive neurons and synaptic spines in the PFC, leading to the preferential use of PCP over MK-801 at inducing symptoms of schizophrenia for study (Jones et al., 2011; Mouri et al., 2007). The effects of a chronic, as opposed to an acute,

exposure of PCP also better represents facets of schizophrenia (Jones et al., 2011; Mouri et al., 2007).

Neonatal PCP administration on PD 7, 9, and 11 has found to induce lasting behavioural changes in adulthood, such as locomotor sensitization with PCP challenge, deficits in prepulse inhibition, and enduring cognitive deficits (Jones et al., 2011). All behavioural deficits were reversed with atypical antipsychotic drugs but data presented on this neonatal PCP model has been inconsistent to date (Jones et al., 2011). Interestingly, administration of MK-801 on PD 7-10 or 20 was found to induce these same deficits, symptoms of which were not found with adult MK-801 administration (Jones et al., 2011). Despite promise in the neonatal pharmacological models of schizophrenia, administration of MK-801 or PCP both cause a significant decrease in body weight across development, an unlikely feature of schizophrenia (Jones et al., 2011). The predictive validity must also be further explored in these perinatal pharmacological treated models of schizophrenia. This perinatal PCP or MK-801 model may, however, be a preferred choice over the adult PCP or MK-801 treated model to study the neurodevelopmental hypothesis of schizophrenia.

1.7.3. Neurodevelopmental models

Animal models testing this hypothesis include early lesioning of select brain regions (i.e. hippocampus. PFC), prenatal exposure to specific viruses (i.e. influenza, Borna virus), as well as prenatal hypoxic or ischaemic insults (McGrath et al., 2003). Unlike pharmacological models directly perturbing specific neurotransmitter systems, or

genetic models targeting specific genes of interest, neurodevelopmental animal models were first created to test the various hypotheses on the origins of schizophrenia (Tseng et al., 2009). The Lipska-Weinberger group first conceived the neurodevelopmental animal model in the early 1990s in attempt to capture the prominent aspects of schizophrenia unaddressed by pharmacological animal models of the disease (Tseng et al., 2009; Jones et al., 2011). Neurodevelopmental models of the disease have been able to encompass a wider array of clinical and neurobiological features of the disease in comparison to traditional animal models of the disease (Tseng et al., 2009).

Neonatal damage of the hippocampus in rats and monkeys, otherwise known as the neonatal ventral hippocampal lesion (NVHL) model is the most thoroughly characterized and published neurodevelopmental animal model for schizophrenia (Tseng et al., 2009; Lipska, 2004). In the rat NVHL animal model, excitotoxin ibotenic-acid is infused locally into the hippocampus of seven-days old rat pups (PD 7), seeing as this period is comparable to fetal hippocampal development during the second and third trimester of human pregnancy (Tseng et al., 2009; Jones et al., 2011). The ventral hippocampus corresponds to the anterior hippocampus in humans, of which anatomical abnormalities have been found in human patients with schizophrenia (Tseng et al., 2009; Lipska, 2004). Excitotoxic lesions into the hippocampus during neonatal development have been found to also disrupt surrounding innervated cortical and subcortical regions, such as the PFC and ventral striatum, as well as the various associated neurotransmitter systems (Tseng et al., 2009; Lipska, 2004). In the NVHL model, developmental loss of the ventral hippocampal excitatory neurons could trigger post-synaptic structural and

molecular rearrangements, leading to dysregulation of the mesocorticolimbic dopamine system and impaired prefrontal cortical functioning (Tseng et al., 2009). At pre-pubertal stages, lesioned animals were found to be less social than control animals (Lipska, 2004). At post-pubertal stages, there was an emergence of hyperactivity, enhanced sensitivity to stimulants (i.e. MK-801, PCP), deficits in prepulse inhibition, impaired social behavior, and working memory problems (Lipska, 2004). Findings suggest aberrant dopamine-GABA signalling in the cortex, including a reduction in dopamine release, GAD_{67}, BDNF, and altered transcription factors c-fos (Tseng et al., 2009; Lipska, 2004). Despite the fact that the NVHL model does not directly target a particular pathway in its method of induction but rather induce a broad non-specific ablation of hippocampal neurons, many of the published molecular changes in the NVHL model parallel findings in human post-mortem samples (Tseng et al., 2009; Lipska, 2004).Some studies suggest there may be a genetic component influencing behavioural anomalies in the NVHL model. DISC-1 is highly present in the hippocampus, and is essential for hippocampal neurite outgrowth and neurogenesis. The NVHL model can essentially be considered a crude model of genetic “lesion” in which maturation events related to DISC-1 in the hippocampus is severely interrupted (Tseng et al., 2009). The NVHL animal model produces behavioural abnormalities, which closely represent symptoms of the positive, negative, and cognitive symptom classes (Tseng et al., 2009). However, discrepancies also exist between the NVHL model and the human disorder, such as an increase in prefrontal cortical synaptic densities, branches, and dendritic length (Lipska, 2004). The predictive validity of the NVHL model has been demonstrated with the ability of antipsychotic drugs to attenuate

the lesion-induced schizophrenia-like behaviours (Lipska & Weinberger, 1994; Tseng et al., 2009).

Other types of neurodevelopmental models have also been described over the years. The maternal immune activation (MIA) animal model is an animal model of maternal viral infection, which has been developed based on the hypothesis that maternal immune response to a viral infection can influence fetal brain development and increase associated risk for schizophrenia (Ozawa et al., 2006). A synthetic double stranded RNA polyriboinosinic-polyribocytidilic acid (poly I:C) is administered into pregnant dams to mimic a viral infection between the periods of embryonic days 12 to 17 (E 12 to E17) (Ozawa et al., 2006). The presence of an infection has been shown to increase the expression levels of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-8 in maternal serum levels, which is likely to interfere with normal fetal brain development (Ozawa et al., 2006). Moreover, prenatal injections of inflammatory cytokines associated with viral infection, including interleukin-I beta, interleukin-6, and TNF-α inhibited the development of dendrites in embryonic cortical neurons in a consistent manner with the neuropathology of schizophrenia (Ozawa et al., 2006). Adult offspring of these infected dams develop maturation-dependent schizophrenia-like symptoms, such as deficits in prepulse inhibition, hyperlocomotor activity, and deficits in novel object recognition (Ozawa et al., 2006). Immune activation can also be provoked by the injection of various other viruses, including the influenza virus, and lipopolysaccharide (LPS) (Ozawa et al., 2006; Patterson, 2009; Meyer & Feldon, 2010; Shi, Fatemi, Sidwell, & Patterson, 2003). Due to the heterogeneity in

symptomology of both schizophrenia and autism, the MIA animal model has been used to study both disorders. The outcome of disease manifestation (i.e. schizophrenia or autism) is closely tied with the severity of MIA induced, the dose of the injected virus, and the genotype of the mother and fetus; this further supports the role of a genetic predisposition in the development of schizophrenia (Patterson, 2009).

Other epidemiology-derived environmental stimuli have similar been used to test the causality of schizophrenia in additional neurodevelopmental animal models of the disease (Meyer et al., 2010). Psychological stress (i.e. restraint, electric foot shock, swimming in cold water, loud white noise, and other unpredictable stress factors), glucocorticoid treatment, protein deprivation, vitamin D deficiency, and obstetric complications like Caesarian section and perinatal/postnatal hypoxia are tested in other neurodevelopmental models (Meyer et al., 2010). The long-term consequences of prenatal and perinatal exposures arising from a broad range of epidemiology factors capture the origins and spectrum of abnormalities pertinent to schizophrenia (Meyer et al., 2010). Interestingly, the observed changes during adolescence are not connected to the surge and fluctuations in hormones at this period in time, as the induced schizophrenia-related abnormalities were observed even in animals depleted of gonadal hormones before puberty (Tseng et al., 2009; Lipska et al., 1994; Lipska, 2004).

In general, developmental models of schizophrenia are difficult to produce due to maternal rejection of pups and premature pup death. Moreover, symptoms can be difficult to replicate owing to the complexities surrounding the procedure and precision required to induce the specific brain insult at a critical window of opportunity (McGrath et al.,

2003). Not only is the nature of an early lesion imprecise, a disruption of early brain developmental processes can underlie a whole range of adverse neurological outcomes which can lead to a spectrum of mental disorders and outcomes (i.e. epilepsy, attention deficit disorder, dyslexia, intellectual handicap) (McGrath et al., 2003). Despite of this innate weakness, the heuristic value of this neurodevelopmental hypothesis of schizophrenia and the use of neurodevelopmental animal models of schizophrenia has well withstood the test of time (McGrath et al., 2003).

1.8. Gene Knock-Down Technology

Gene manipulation is often used in animal models to better study the functions of specific genes of interest within the organism. Knock-out, knockdown, or knock-in of gene targets are common strategies to isolate function of a specific gene both *in vitro* and *in vivo*. These methods can be utilized to target specific interactions, manipulate gene expression, and identify gene functions.

1.8.1. Antisense Deoxyoligonucleotides (AS)

In comparison to the use of other gene manipulation models, such as the widely used knock-out animal, the utilization of the AS technology is preferred due to its ease in execution, low cost, high probability of successful knockdown, and high specificity for the gene target (Kurreck, 2003). Use of AS as compared to gene knockout technology also offers a higher survival rate in manipulated animals (Kurreck, 2003). AS are useful agents developed to modify the expression of highly specific target gene(s) in a sequence-

specific manner (Kurreck, 2003). Sequences usually consist of 15-20 nucleotides complementary to the target mRNA, and function by inhibiting the translation of the target mRNA into protein (Kurreck, 2003). An online database search (Pubmed BLAST) will determine the specificity of the AS sequence to the target mRNA of interest. As in any study, the appropriate controls must also be used to ensure that the effects observed are a result of specific antisense inhibition of the gene and not simply the presence of an external material. Sense or mismatch deoxyoligonucleotide sequences are commonly used as options for negative controls (Kurreck, 2003).

There are two mechanisms by which designed AS induce the inhibition of gene expression. AS can activate RNase H, an enzyme which cleaves the RNA moiety of the DNA-RNA heteroduplex though RNase H-mediated degradation of RNA.The AS sequence can also inhibit translation through steric blockade of the ribosome (Kurreck, 2003; Scherer & Rossi, 2003). In order to effectively reduce of gene translation, the designed oligonucleotide sequence must: (1) specifically and easily access the target RNA, (2) be protected against cellular degradation, and (3) have correct cellular uptake and intracellular localization (Kurreck, 2003). Natural phosphodiester oligodexoyoligonucleotides are rapidly degraded by nucleases *in vitro* and in *vivo*. Thus, scientists have designed several modification strategies to strengthen base-pairing and stabilize the DNA-RNA duplex in the presence of nucleases. Modifications include analogs with unnatural bases, modified sugars at the 2' ribose position, or altered phosphate backbones such as added phosphothioates to the bases (Kurreck, 2003). Appropriate delivery methods must also be used to promote the internalization and

uptake of AS into cells for *in vitro* and *in vivo* delivery. In order to maintain efficient cellular uptake and sustained gene inhibition, receptor-mediated endocytosis or transfection reagents can be employed to encapsulate nucleic acids and promote the crossing of DNA across membrane lipids (Kurreck, 2003; Achenbach, Brunner, & Heermeier, 2003). Interesting, efficient DNA uptake has also been observed *in vivo* following direct intracranial injection into the brain region of interest (Kurreck, 2003; Dyck et al., 2011).

2.0. Hypotheses and Rationales

The overall objective of this project was to investigate the role of decreased synapsin II expression levels during critical periods of neurodevelopment and its effect on the pathophysiology of schizophrenia. In pursuit of this objective, a combination of behavioural and neurochemical analyses were utilized. In addition, efficacy of the atypical antipsychotic, olanzapine, to attenuate alterations pertinent to schizophrenia was examined. Our overarching hypothesis was that a specific neurodevelopmental knockdown of synapsin II in the mPFC will cause the production of lasting schizophrenia-like behavioural and neurochemical changes into adulthood.

2.1. Synapsin II knockdown on postnatal day (PD) 7 will not cause lasting schizophrenia-like changes into adulthood.

Rationale: Brain development is a continuing process, but only insults during specific critical periods of development can have a profound influence on developmental

processes and disease production. Examples can be found with peak vulnerability periods for viral infections during the second trimester of pregnancy, or the harmful effects of maternal malnutrition during the first trimester of pregnancy in humans. Further evidence for a critical stage of development is found when tetradoxin injections into the hippocampus of the neonatal rat, but not the adult rat, caused permanent brain alterations representative of schizophrenia (Lipska, 2004). In addition, NMDA antagonist treatment (i.e. PCP or MK-801) during the developmental period, as opposed to adulthood, can also have a more permanent effect on the development of schizophrenia-like behaviours in the rat post-drug cessation (section 1.7.3.).

Lesions to the ventral hippocampus at the neonatal stage have been established to induce schizophrenia-related behaviours in adulthood. Similar behavioural abnormalities have also been observed following lesions to the mPFC by ibotenic acid during developmental stage PD 7 (Weinberger et al., 2001; Tseng, Amin, Lewis, & O'Donnell, 2006; Lipska, al Amin, & Weinberger, 1998; Flores, Silva-Gomez, Ibanez, Quirion, & Srivastava, 2005; Schwabe, Klein, & Koch, 2006; Schneider & Koch, 2005). Although results implicate both the mPFC and hippocampus in the development of schizophrenia-like behaviours, previous knockdown studies in adult rats from our laboratory have shown that schizophrenia-like behavioural changes only result from depleted synapsin II expression levels in the mPFC, but not the hippocampus or cerebellum, of the rat (Dyck et al., 2011).

The following study investigated the early developmental age of susceptibility at which ablation of synapsin II within the mPFC would induce lasting schizophrenia-like

behavioural alterations into adulthood. PD 7 marks the beginning of synapsin II expression in the neocortex of the developing brain (Zurmohle, Herms, Schlingensiepen, Brysch, & Schlingensiepen, 1996). In order to better understand the critical periods of synapsin expression, neurogenesis, and synaptic plasticity, the following study determined if a one-time PD 7 knockdown of synapsin II in the mPFC was sufficient to induce lasting schizophrenia-like behavioural abnormalities at pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) adult stages of adulthood (**Figure 9**). We hypothesized that a one-time knockdown of synapsin II during the initiation of synapsin II expression may be insufficient to cause lasting alterations in schizophrenia-like behaviours as compared to studies in Objective 2.2 targeting synapsin II knockdown during a maximal synapsin II expression period of PD 17-23. This lack of penetrance in a one-time gene knockdown may be attributed to the compensation of synapsin II levels immediately following the PD 7 knockdown period (**Figure 8**) (Zurmohle et al., 1996).

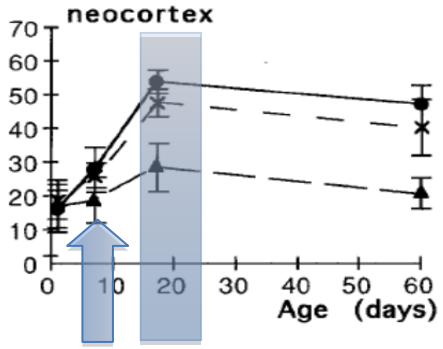


Figure 8: mRNA expression of synapsin Ia, Ib, and IIa is indicated by filled circles, crosses, and filled triangles, respectively (Zurmohle et al., 1996). Expression levels of synapsins are indicated for the region of the neocortex, which later develops into the cerebral cortex. Densitometric readings are indicated on the y-axis and age (in days) is indicated on the x-axis. As indicated by the shaded regions of the graph, PD 7 marks the initiation of synapsins expression while maximal expression of synapsins is indicated between PD 17-23. Graph has been modified from Zurmöhle *et al*. (Zurmohle et al., 1996).

2.2. Synapsin II knockdown on postnatal day (PD) 17-23 will induce lasting schizophrenia-like behavioural changes which will persist into pre-pubertal and post-pubertal stages of adulthood.

Rationale: In neurodevelopmental models of schizophrenia, the insult often presents itself early on in life far prior the onset of symptoms. This is similar to the clinical manifestation of symptoms, whereby neurodevelopmental insults during prenatal or perinatal periods are found to influence clinical onset of symptoms long after insult cessation. Adolescence is a period in development in which many changes occur within the body and brain; these changes may likely trigger the expression and vulnerability of schizophrenia (Walker & Bollini, 2002). A number of things can happen during this period of adolescence which can, either inclusively or exclusively, act as contributing factors precipitating disease onset. Both hormonal changes and brain development during adolescence have been thought to form the basis for behavioural changes such as symptom manifestation of schizophrenia (Walker et al., 2002; Lipska et al., 1994; Tseng et al., 2009; Lipska, 2004). If schizophrenia-related abnormalities in this synapsin II neonatal animal model of schizophrenia developed as a function of hormonal changes during puberty, we expect behavioural abnormalities to lie stagnant at pre-pubertal stages, and only manifest at post-pubertal stages. If, however, schizophrenia-related abnormalites developed as a function of brain development, we expect behavioural abnormalities to manifest both at pre-pubertal and post-pubertal stages, since PFC synaptic pruning and mPFC maturation occurs pre-puberty from PD 30 onwards (**Figure 1**). Studies by Lipska *et al.* suggested that gonadal hormones may not be involved in the development of

schizophrenia-like abnormalities, therefore, we expected to find the latter of the above stated scenarios in the production pattern of schizophrenia-like behavioural abnormalities (Lipska et al., 1994; Tseng et al., 2009; Lipska, 2004).

Synapsins are integral to synaptic stability and neurotransmission. Knowing its crucial importance in synaptogenesis and the maintenance of neurotransmission, we sought to determine if there is any neurodevelopmental value in synapsin II gene interruptions. Since synaptogenesis and synaptic plasticity are important aspects of neurodevelopment, subtle disruption of synapsin II in neonates during a crucial developmental period can be conceived to interfere with normal brain development and maturation, causing miswiring in neural connections and resulting behavioral abnormalities pertinent to schizophrenia. A knockdown of synapsin II levels during adulthood has been previously found induce an array of symptoms representing that of the positive, negative, and cognitive domains of schizophrenia (Dyck et al., 2011). In this study, we proposed that a knockdown of synapsin II during a critical stage in development will induce similar, if not more severe, schizophrenia-like symptoms following the maturation of these neonatal knockdown animals. In the adult synapsin II knockdown animal, abnormalities in behaviour and dysregulation in neurotransmission were found to be non-permanent and ceased upon the normalization of synapsin II expression levels. Unlike the adult synapsin II model, changes pertaining to this neurodevelopmental gene knockdown of synapsin II are expected to be permanent and persist into adulthood following the cessation of synapsin II knockdown.

Since maximal expression of synapsin II occurs between PD 17-23, synapsin II

knockdown was performed for 7 consecutive days on PD 17-23 via the use of bilateral guide cannulas (**Figure 9**) (Zurmohle et al., 1996). This temporal pattern of synapsin II gene expression coincides with neurogenesis and synaptogenesis during PD 5-20 (Devoto & Barnstable, 1989). We determined if a 7-day knockdown of synapsin II in the mPFC during this period of maximal synapsin II gene expression was sufficient to induce lasting schizophrenia-like abnormalities into pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages (**Figure 9**). Behavioural symptoms were examined in detail for their resemblance to the various symptoms classes in schizophrenia, and were expected to parallel the abnormalities observed in established animal models of the disease as well as in the human clinical population. We hypothesized that a temporal, but selective, 7-day knockdown of synapsin II during PD 17-23 was sufficient to trigger the manifestation of permanent behavioural changes, as indicated by (1) an increase in locomotor activity, (2) a reduction in social interaction, and (3) deficits in prepulse inhibition. Moreover, we hypothesized that these neurodevelopmental synapsin II knockdown-induced abnormalities in behaviour (4) will persist into pre-pubertal and post-pubertal stages of adulthood.

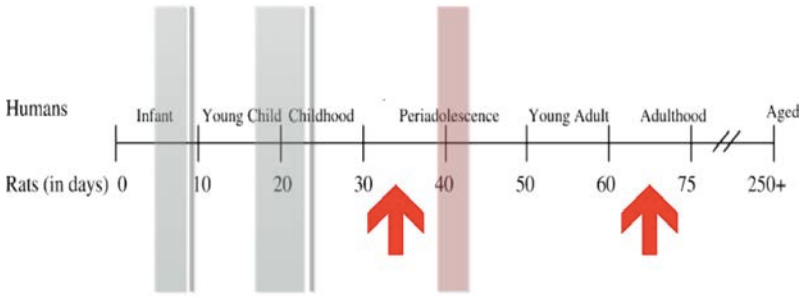


Figure 9: A relative comparison of ages and stages of development between human and rat species (Andersen, 2003). As indicated in the above study design, acute synapsin II knockdown was induced at either PD 7 or PD 17-23. Animals were then allowed to develop normally into adulthood. Behaviour and neurochemical changes were assessed at pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages, as indicated by the arrows. Puberty occurs between PD 35-55 (Gear et al., 2007; Zemunik et al., 2003). Patients with schizophrenia typically manifest symptoms during late teens or early adulthood (16-25 human years: 10-25 years for men, 25-35 for women), but premorbid symptoms may manifest during middle age or at a later age. Rare cases of schizophrenia may also develop in young children and adolescents. Figure has been modified from Andersen *et al.* (Andersen, 2003).

2.3. Synapsin II knockdown on postnatal day (PD) 17-23 will induce lasting schizophrenia-like neurochemical changes within the medial prefrontal cortex which will persist into pre-pubertal and post-pubertal stages of adulthood.

Rationale: Previous studies from our laboratory have found a knockdown of synapsin II in the mPFC of the adult rat to cause the production of dopamine-related and glutamate-related behavioural abnormalities (Dyck et al., 2011). Moreover, knockdown of synapsin II in the mPFC induced a dysregulation in the glutamate and GABA, but not dopamine, neurotransmitter systems (Dyck et al., 2011). Brain interruptions during specific periods of development can often cause lasting neurochemical brain alterations, which may manifest into behavioural abnormalities in adulthood. Manifested symptoms in schizophrenia are thought to result from a developmental insult and the lack of compensation over time.

After establishing a causal role of synapsin II knockdown in the production of behavioural abnormalities typical of schizophrenia (section 2.2), we sought to determine the specific neurochemical consequences of a reduction in synapsin II expression levels. Due to a lack of compensation in synapsin II expression levels immediately following PD 17-23 knockdown, we expected the reduction in synapsin II expression levels to persist into pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages (Zurmohle et al., 1996). We investigated if the synapsin II knockdown-induced neurochemical abnormalities at adulthood reflected a dysregulation in the modulatory (i.e. dopamine), excitatory (i.e. glutamate), and inhibitory (i.e. GABA) neurotransmitter systems within the mPFC (**Figure 4A-B**). Measurement of change in the respective neurotransmitter systems was

determined indirectly through protein estimation of the associated transporters and enzymes: (1) quantification of VGLUT-1 and VGLUT-2 determined the level of change in the glutamate system, (2) quantification of VGAT and $GAD_{65/67}$ determined the level of change in the GABA system, and (3) quantification of VMAT-2 and DAT determined the level of change in the dopamine system. All protein measurements were determined via immunoblotting with use of antibodies specific for the various proteins of interest. Based on findings in adult synapsin II knockdown animals, established models of schizophrenia, and human patients of the disease, we expected to find (1) hypo-glutamate transmission within the mPFC, as measured by reduced protein expression of VGLUT-1, VGLUT-2, and/or synapsin II, (2) possible GABA reductions within the mPFC, as measured by reduced protein expression of VGAT and/or $GAD_{65/67}$, and (3) no change in dopamine transmission as measured by unchanged protein expression of VMAT-2 and DAT. Moreover, we expected these abnormalities in the respective neurotransmitter systems (4) to persist into pre-pubertal and post-pubertal stages of adulthood. We hypothesized that a temporal but specific knockdown of synapsin II during the critical period of PD 17-23 was sufficient to cause lasting reductions in the protein expression of synapsin II, as well as altered mPFC glutamate and GABA systems into pre-pubertal and post-pubertal stages.

2.4. Atypical antipsychotic, olanzapine, treatment will attenuate both behavioural and neurochemical alterations induced by developmental alterations of synapsin II expression levels.

Rationale: The use of antipsychotic drugs can often attenuate behavioural and neurochemical changes associated with schizophrenia, both in animal models of the disease and in clinical populations. Different drugs vary in their capacity to target specific behaviours, brain regions, and receptor targets implicated in schizophrenia. Previous studies from our laboratory indicated that the binding of stimulatory and excitatory dopamine receptors can cause differential changes in synapsin II expression; agonism of the excitatory D_1 receptor and antagonism of the stimulatory D_2 receptor were both found to promote an increase in synapsin II expression (Chong et al., 2006). Antipsychotic drug use was also found to attenuate the reduction of synapsin II levels in the dorsolateral PFC of human post-mortem samples (Tan et al., 2014). In the previously founded adult synapsin II knockdown model, treatment with atypical antipsychotic drug olanzapine, but not typical antipsychotic drug haloperidol, was found to be effective at attenuating both the behavioural and neurochemical alterations associated with synapsin II knockdown. Our laboratory has previously established the efficacy of antipsychotic drugs on (1) the normalization of synapsin II expression levels, and (2) the attenuation of positive, negative, and cognitive behavioural abnormalities associated with adult synapsin II reductions. In addition, given olanzapine's broad spectrum of receptor targets within the mPFC, olanzapine treatment was established to be effective at (3) attenuating the associated dysregulation in the glutamate and GABA neurotransmitter systems, following adult synapsin II knockdown (Dyck et al., 2011; Guest et al., 2010).

The predictive validity of this newly developed neurodevelopmental synapsin II knockdown model was examined in the ensuring study objective. Experiments were

designed to examine the therapeutic ability of antipsychotic drug, olanzapine, to reverse the induced schizophrenia-like behavioural and neurochemical abnormalities resulting from the neurodevelopmental knockdown of synapsin II. We hypothesized that an acute treatment with atypical antipsychotic drug, olanzapine, will (1) normalize alterations in synapsin II protein expression levels, (2) attenuate the schizophrenia-related behavioural abnormalities (as described in section 2.2.), as well as (3) normalize dyregulations in the mPFC glutamate and/or GABA systems resulting from neurodevelopmental synapsin II knockdown (as described in section 2.3.).

2.5 Significance

Despite decades of research into the design of therapeutic options for schizophrenia, existing treatments for this disease merely manage the symptoms in afflicted patients. Further investigation into the underlying mechanisms will supplement knowledge of the disease and effectively fuel the design of better targeted therapeutics. The link between disruptions in synapsin II gene expression in the etiology of schizophrenia has been established through studies from our laboratory, but findings regarding this causal link have otherwise been limited. Previous studies in the neonate have investigated the effects of a global synapsin II depletion, but a specific and temporal interruptions of synapsin II expression during development has not been previously explored (Dyck et al., 2009; Dyck et al., 2011; Dyck et al., 2007). Further investigation into the neurodevelopmental role of synapsin II in schizophrenia, as well as the possible delineation of altered neurotransmitter systems within the mPFC, can elucidate the role of

synapsin II during neurodevelopment and the possible causative element of this phosphoprotein in disease development. To our knowledge, this is the first study investigating the behavioural and neurochemical consequences of a temporal developmental interruption of synapsin II in the mPFC. In order to further establish this disease model as a valid animal model of schizophrenia, research questions have been designed to specifically address the face, construct, and predictive validities of this newly developed synapsin II neurodevelopmental disease model. *Face validity* refers to how phenotypically similar the present animal model is when compared to the behavioural and physiological responses monitored in human subjects with the disease. *Construct validity* refers to the common etiology and mechanisms underlying the present animal model and the human disorder. *Predictive validity* refers to the ability of the present animal model to respond to similar medications as human patients with the disorder. Future studies will elucidate additional behaviours and neurochemical changes in this newly developed animal model. However, results thus far demonstrate all three validities, authenticating the use of this animal model of schizophrenia for further mechanistic and therapeutic studies.

Findings from this study heavily supplement the current understanding of PFC hypoglutamatergic function in schizophrenia. By understanding synapsin II knockdown-induced hypoglutamatergic function in the mPFC, as well as the effects such hypoglutamatergic function can have on other neurotransmitter systems, researchers come closer to understanding the pathogenesis of schizophrenia and the mechanisms underlying symptom manifestation. There is tacit understanding of an existing genetic

predisposition which, together with the brain interruptions during the stated critical periods, influence the occurrence of disease outcome (McGrath et al., 2003). Results emphasize the importance of synapsin II in the generation of mental illness and provide insight on how the differing classes of neurotransmitter systems interact in heavy dependence on synapsin II expression. With such a central role influencing the integrity of the various neurotransmitter systems, any compromise of synapsin II expression levels in the mPFC can bear detrimental outcomes for disease development. This study further suggests that slight alterations of synapsin II expression levels only during crucial stages of development is sufficient to cause the production of lasting schizophrenia-like abnormalities into both pre-pubertal and post-pubertal stages. In this novel animal model of schizophrenia developed in our laboratory, the genetic and neurodevelopmental factors influencing synaptic expression in the PFC becomes apparent when this brain region comes “online” with the natural process of brain development, staging the production of schizophrenia-like abnormalities in early adulthood. Alterations in synapsin II expression is found pivotal to the generation of schizophrenia-like abnormalities, the development of which is found to correlate with the maturation stages of the PFC.

For the first time, I have demonstrated a link between neurodevelopmental prefrontal cortical synapsin II expression levels and schizophrenia. Within this newly developed animal model of schizophrenia which I have developed, I indicated hypoglutamatergic activity within the PFC as the causal mechanim to the induction of schizophrenia-like behavioural abnormalities. This synapsin II developmental animal model has been and will continue to be an invaluable tool for studying schizophrenia.

Importantly, results from this study provide new strategies for elucidating the mechanisms of this disorder, as well as clues for the development of effective therapeutics devoid of associated side effects. This discovery may platform the design of diagnostic tools used to assess the risk of an individual towards developing a mental illness prior onset, and stage the development for a pharmacogenomics approach towards treating mental illnesses. Improved comprehension of the disease will ultimately progress current therapeutic measures from a symptomatic approach to the design of more specifically targeted therapeutic compounds for prodromal treatment prior the onset of disease.

3. METHODOLOGY

3.1. Animals

Pregnant female Sprague Dawley rats at 13-15 days gestation will be commercially obtained from Charles River. All animals were housed in the Central Animal Facility at McMaster University and cared for under the Canadian Council for Animal Care guidelines. Animals were housed individually in standard cages in a room maintained at 22°C and kept on a 12 hr light/dark cycle beginning at 7am/7pm. Rats were given *ad libitum* access to food pellets and water. Once born, pups were left to nurse with their mothers and were weaned on PD 24.

3.2. Antisense sequences and preparation

As with the experiments previously performed in the adult rats, synapsin II knockdown in the mPFC of the neonate will be induced using AS sequences designed to

block cellular mRNA and inhibit translation of synapsin II (**Table 1**). All nucleotides were capped with phosphorothioates to prevent nuclease destruction. The sense oligonucleotide sequence for the same synapsin II coding region will serve as a negative control (Dyck et al., 2011; Morishita et al., 1993). Oligonucleotides were dissolved in nuclease-free water (NFH_2O) to a final concentration of 1 mM. The solution is then filtered through sterile 0.22 µm Acrodisc® Syringe Filters with HT Tuffryn® Membrane (Pall Corporation, Ann Arbor, MI, USA) prior infusion into the brain.

	Nucleotide Sequence
Antisense (AS)	A*G*T*T*C*A*T*C*A*T*C*T*G*G*C*T*T*G*A*G*G*G*A
Sense	T*C*C*C*T*C*A*A*G*C*C*A*G*A*T*G*A*T*G*A*A*C*T

Table 1: AS and sense deoxyoligonucleotides for the synapsin II gene were synthesized and purchased from Integrated DNA Technologies (IDT). All nucleotides were capped with phosphorothioates to prevent nuclease degradation *in vivo*. * indicate added phosphorothiotes. The synapsin II AS oligonucleotide binds to the mRNA sequence of synapsin II and inhibits the translation of synapsin II. The sense oligonucleotide sequence for this same coding region served as a negative control (Morishita et al., 1993).

3.3. Surgery

(1) PD 7 Synapsin II Knockdown

On PD 7, pups were anesthetized via hypothermia and mounted on a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with a fitted stereotaxic neonatal rat adaptor (Stoelting Co., Wood Dale, IL). During this time, animals underwent a one-time bilateral infusion (1 μl/minute, 2 ul of 1 mM solution/side, 2 nmole/side) using a Hamilton syringe (Hamilton, Reno, NV) into the mPFC (coordinates A/P +2.5 mm, M/L ±1.0 mm and D/V -3.0 mm relative to bregma) (Schneider et al., 2005). Although smaller and less developed, the mPFC of the rat is functionally analogous to the dorsolateral PFC of the human, a region which is heavily implicated in the pathophysiology of schizophrenia (DeVito 2010, 17: 161-167). Treatment groups (n=3-6/group) for the investigation of a one-time PD 7 knockdown were as follows: **Group A**: artificial cerebrospinal fluid (aCSF) and **Group B**: synapsin II AS.

(2) PD 17-23 Synapsin II Knockdown

On PD 15, a separate group of pups were anesthetized with gaseous anesthetic (Isofuorane, Pharmaceutical Partners of Canada Inc, Richmond Hill, ON) and mounted on a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with a fitted stereotaxic neonatal rat adaptor (Stoelting Co., Wood Dale, IL). During this time, animals were implanted with a bilateral guide cannulae (HRS Scientific, Anjou, QC) into the mPFC (coordinates A/P +2.6mm, M/L ±1.0mm and D/V -3.0mm relative to bregma) (Schneider et al., 2005). Starting on PD 17, neonates underwent 7 consecutive days (PD 17-23) of bilateral infusion 1 μl/minute, 4 ul of 1 mM solution/side, 4 nmole/side).

Treatment groups (n = 6-42/group) were as follows: **Group A**: aCSF; **Group B**: sense sequence, and **Group C**: synapsin II AS. On PD 24, bilateral guide cannulas were removed and incision was glued together with tissue adhesive (3M VetBond). Post-recovery, pups were returned to their respective mothers. Pups were weaned on PD 25 and left to mature until behavioural and neurochemical testing at pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages.

3.4. Drug Preparation

5 mg, 10 mg, and 15 mg Zyprexa® (olanzapine) tablets were obtained from Eli Lilly (Scarborough, ON). Tablets were crushed with a pestle and mortar in 3 x 10ml additions of 100% ethanol. Solution was then centrifuged at 10 000 rpm for 10 min. The clear yellow supernatant was extracted and left to dry on an even glass surface, leaving behind a yellow powder (pure olanzapine) upon evaporation. This process was repeated for all 3 lots of olanzapine, and the individual lots were combined to form one uniform stock of olanzapine. Purity of olanzapine was then determined via high-performance liquid chromatography (HPLC).

3.4.1. HPLC - Determining Drug Purity

Samples were run on a Nova-Pak C18 column (Waters, Mississauga, ON) with 4 µm particle size, 3.9 mm internal diameter and 150 mm in length. Composition of the mobile phase included 20 mM ammonium acetate in water:acetonile:methanol in a ratio of 30:35:35 (v/v/v) at a constant flow rate of 0.8 mL/min. Prior mixing with the mobile

phase, ammonium acetate solution was filtered in the hood using a sterile Nalgene® Bottletop sterile filter unit (500 ml, 0.45 µm filter pore size) (Fisher Scientific, Nepean, ON). A run time of 10 min was provided although separation olanzapine peaks were observed at 3-4 min. The detection was carried out at 227nm. An injection volume of 10 µl was used for all standards and samples. A total of 3 injections/sample was run.

A standard of olanzapine with 98% HPLC purity was obtained from Sigma (Oakville, ON). Standard solutions of concentrations 0.2 mg/ml, 0.1mg/ml, 0.05 mg/ml, 0.02 mg/ml, 0.01 mg/ml, 0.004 mg/ml, 0.002 mg/ml, and 0.001 mg/ml were created in 100% methanol solution. A standard curve was constructed with the obtained standard peaks obtained and a linear regression of $R^2 > 0.99$ was considered a good fit. Sample stock solutions of 0.2 mg/ml, 0.1 mg/ml, and 0.05 mg/ml was created in 100% methanol. Obtained sample peaks were then plotted against the standard curve to quantitatively determine the percentage of pure olanzapine within the samples of extracted olanzapine tablets. All standard and sample dilutions were filtered using sterile 0.22 µm Acrodisc® Syringe Filters with HT Tuffryn® Membrane (Pall Corporation, Ann Arbor, MI, USA).

3.4.2. Drug Treatment

2 mg/ml stock solution of olanzapine was created in a vehicle composition of 0.8% acetic acid in 0.9% NaCl solution, and was neutralized to a pH of 6 with 10 N NaOH (approximately 3 ul) prior intraperitoneal (i.p.) injection into animal. Prior the pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages of behavioural and neurochemical assessments, a subgroup of **Group C** from **section 3.3.** of PD 17-23

synapsin II AS-treated animals was randomly selected and treated with daily 7.5 mg/kg (i.p.) of olanzapine (Eli Lilly) for 8 consecutive days (Dyck et al., 2011). A saline-treated control was also used. Treatment groups were as follows: **Group Co**: synapsin II AS + acute olanzapine injection, and **Group Cs:** synapsin II AS + saline injection.

3.5. Behavioural Testing

Behavioural testing was performed at pre-pubertal (PD 32-36) and post-pubertal stages (PD 65-70) to assess the presence of schizophrenia-like behavioural abnormalities. Baseline behavioural testing was not performed due to the nature of the study. Behavioural testing assessed the manifestation of symptoms related to schizophrenia, namely: locomotor hyperactivity, social withdrawal, and deficits in prepulse inhibition. Details and results for behavioural tests are described below.

3.5.1. Locomotor Activity

Hyperlocomotor activity in preclinical animal models of schizophrenia reflect increased levels of dopamine in the subcortical regions of the brain (i.e. striatum, NAc), which is a consistent finding in the brains of patients with schizophrenia (Carlsson et al., 2001; Seeman, 2011; Laruelle et al., 2003; Kapur et al., 2000). This augmented increase in dopamine levels resulting from hyperlocomotion is frequently used as an index with translational relevance to the positive symptoms in humans with schizophrenia (Jones et al., 2011). Rats were placed in computerized cages (Accuscan Instruments, Columbus, Ohio, USA) during their dark/wake cycle for 180 minutes, during which their total

distance travelled was recorded in intervals of 10 minutes (Dyck et al., 2009; Dyck et al., 2011). The initial 30 minutes was considered habituation, with the last 150 minutes deemed as actual activity. Total distance travelled was measured in centimeters (cm).

3.5.2. Social Interaction

Social withdrawal is a key component of the negative symptoms of schizophrenia, and is one of the first indicators of the disease (Dyck et al., 2007). Negative symptoms, such as social withdrawal and blunted affect, can be easily detected in patients with schizophrenia and preclinical animal models (Dyck et al., 2007; Sams-Dodd, 1998a). In this test, two rats of the same treatment group were placed in an arena for 10 minutes and the number and length of active and passive interactions were scored. Methods have been adapted from Sams-Dodd *et al.* and Dyck *et al.* (Sams-Dodd, 1998b; Dyck et al., 2011; Dyck et al., 2012b). The initial 5 minutes was considered habituation, with the last 5 minutes deemed as actual activity.

3.5.3. Prepulse Inhibition

Prepulse inhibition is a measure of sensorimotor gating and cognitive fragmentation (Ludewig, Geyer, & Vollenweider, 2003). This technique is commonly used to measure the sensorimotor gating deficit observed in patients with schizophrenia, by assessing their reflex startle response to a brief stimulus. A weak sub-threshold acoustic stimulus (prepulse; 68dB, 71dB, 77dB) was presented prior a strong acoustic stimulus (pulse; 120 dB). The prepulse stimulus served to inhibit the startle response to

the pulse stimulus. Disruptions in sensory processing and sensorimotor gating have been consistently observed in patients with schizophrenia and in established animal models of the disease (Cannon et al., 2002; Dyck et al., 2007; Braff et al., 2001a; Tenn, Kapur, & Fletcher, 2005). Prepulse startle responses in neurodevelopmental synapsin II knockdown animals were assessed using the SR-Lab Startle Response System (San Diego Instruments, San Diego, CA, USA). Methods were adapted from Tenn *et al* and Dyck *et al.* (Tenn, Fletcher, & Kapur, 2003; Dyck et al., 2011; Dyck et al., 2012b; Tenn et al., 2005).

3.6. Sacrifice and Tissue Dissection

Approximately half of all groups of treated animals (**Groups A, B, Cs, Co**) from PD 17-23 in **Section 3.3.** were sacrificed during pre-pubertal stage PD 35, and the rest of the animals in the treatment groups were sacrificed during post-pubertal stage PD 70. Select animals (n = 1-2) were also randomly chosen to be sacrificed on PD 24 from each treatment group to verify infusion site and determine the efficacy of synapsin II knockdown. Animals were heavily anesthetized with Isofluorane (Pharmaceutical Partners of Canada Inc.) and rapidly decapitated. Brains were quickly removed and the mPFC was dissected on ice. Tissues were stored at -80°C until further use.

3.7. Protein Isolation and Quantification

The mPFC was hand homogenized in Tris-EDTA buffer (50 nM Tris, 1nM EDTA, pH 7.4) with protease inhibitor for 10 sec. Protein was then further broken down

through sonication for 5 sec. Protein concentration was determined using a Bradford Assay with the Bio-Rad Protein Assay reagent (Bio-Rad, Mississauga, ON) and a CU-640 spectrophotometer (Beckman-Coulter, Mississauga, ON). Samples optical density (OD) was measured in duplicates at 595 nm. Concentrated samples which fell outside the linear range of the purified bovine serum albumin (BSA) protein standard of 1.2 - 10.0 µg/ml were further diluted with Tris-EDTA buffer (pH 7.4) and OD was re-estimated. Samples were stored at -80°C until further use.

3.8. Immunoblotting

Immunoblotting was performed as previously described by Dyck et al. (Dyck et al., 2011). 15 µg of protein will be separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto 0.22 µm polyvinylidene fluoride (PVDF) membrane. Membrane blots were blocked with 5% milk bufferin Tris Buffered Saline with Tween® 20 (TBS-T). Blots were incubated with the respective primary (overnight, 4°C) and secondary antibody (1.5-2 hours, room temperature) in TBS-T (**Table 2**). Visualization of protein bands were achieved with enhanced chemiluminscence reagents (Amersham Biosciences, Pittsburgh, PA) and membranes exposed to Kodak Biomax XAR film (PerkinElmer Life Sciences). Protein bands were analyzed using the NIH ImageJ program. Concurrent probing forthe housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), accounted for variations in sample loading and allowed for the normalization of the respective protein bands of

interest (**Table 2**). Concentrations for GAPDH antibody (primary GAPDH antibody of 1:20 000; secondary anti-mouse antibody of 1:10 000) are provided in **Table 3**.

3.8.1. Antisense Specificity

Estimation of synapsin II protein levels can ensure specific, accurate, and effective knockdown of synapsin II in the mPFC. To ensure that the designed synapsin II AS deoxyoligonucleotide sequence is specific to and effective at inducing synapsin IIa and IIb knockdown, immunoblotting experiments were performed with synapsin II-specific antibodies (primary synapsin II antibody of 1:2500; secondary anti-rabbit antibody of 1:5000) (**Table 2**).

3.8.2. Neurotransmitter Systems

Dysregulation of implicated neurotransmitter systems (i.e. dopamine, glutamate, GABA), in the mPFC of the brain were be determined using immunoblotting. Fluctuations in levels of vesicular transporters can indirectly dictate similar alterations in the uptake of the their relevant neurotransmitters. Antibody concentrations are indicated in **Table 2**.

PRIMARY ANTIBODY		
	Antibody	**Concentration**
Synaptic Protein	Synapsin II	1:2,500
Glutamate	VGLUT-1	1:40,000
	VGLUT-2	1:15,000
GABA	VGAT	1:10,000
	$GAD_{65/67}$	1:5,000
Dopamine/Monoamine	VMAT-2	1:5,000
	DAT	1:1,000
Housekeeping	GAPDH	1:20,000
SECONDARY ANTIBODY		
	Antibody	**Concentration**
	Anti-rabbit	1:5,000
	Anti-mouse	1:10,000
	Anti-goat	1:10,000

Table 2: Optimized primary and secondary antibody concentrations used for immunoblotting.

3.8.2.1. Glutamate Transmission

In addition to synaptic proteins, vesicular transporters are also present at presynaptic terminals to help load the respective neurotransmitters into synaptic vesicles (**Figure 5**) (Cesca et al., 2010; Gitler et al., 2008). Reductions in glutamate vesicular transporters (i.e. VGLUT-1, VGLUT-2) have been reported in the dorsolateral PFC of patients with schizophrenia (Dyck et al., 2011; Eastwood & Harrison, 2005; Lewis, Hashimoto, & Volk, 2005; Lewis et al., 2003). Protein estimation of vesicular transporters associated with glutamate-containing synaptic vesicles will provide us will an indirect measure of changes within the glutamatergic system. Concentrations for VGLUT-1 antibody (primary VGLUT-1 antibody of 1:40 000; secondary anti-mouse antibody of 1:10 000) and VGLUT-2 antibody (primary VGLUT-2 antibody of 1:15 000; secondary anti-rabbit antibody of 1:5 000) are provided in **Table 2**.

3.8.2.2. GABA Transmission

Reductions in GABA vesicular transporter (i.e. VGAT) have been reported in the dorsolateral PFC of patients with schizophrenia, but other studies report no change in VGAT expression levels (Dyck et al., 2011; Lewis et al., 2005; Lewis et al., 2003). In addition, GABA synthesizing enzyme (GAD_{67}) has been found to be reduced in the dorsolateral PFC of individuals with schizophrenia in some studies but not others (Lewis et al., 2005; Lewis et al., 2003). Protein estimation of vesicular transporters associated with GABA-containing synaptic vesicles will provide us will an indirect measure of changes within the GABAergic system in this neurodevelopmental animal model of

schizophrenia. Concentrations for $GAD_{65/67}$ antibody (primary $GAD_{65/67}$ antibody of 1:5 000; secondary anti-rabbit antibody of 1:5 000) and VGAT antibody (primary VGAT antibody of 1:10 000; secondary anti-mouse antibody of 1:10 000) are provided in **Table 2**.

3.8.2.3. Dopamine Transmission

VMAT-2 and DAT can also be found in dopaminergic neurons, and alterations will also be estimated via immunoblotting (Hnasko et al., 2010). Protein estimation of vesicular transporters associated with dopamine-containing synaptic vesicles will provide us will an indirect measure of changes within the dopaminergic system. Concentrations for VMAT-2 antibody (primary VMAT-2 antibody of 1:5 000; secondary anti-rabbit antibody of 1:5 000) and DAT antibody (primary DAT antibody of 1:1 000; secondary anti-goat antibody of 1:10 000) are provided in **Table 2**.

3.9. Data Analysis

All behavioural and biochemical data were analyzed by means of one-way ANOVAs with Tukey's post-hoc test (Kozlowski, Connor, Tillerson, Schallert, & Bohn, 2000; Dyck et al., 2012b; Dyck et al., 2011). P-values < 0.05 (95% confidence levels) indicate statistical significance. All statistical analyses were performed using GraphPad 4.0 software (San Diego, CA).

3.9.1. Locomotor Activity Analysis

Total locomotor activity in the 180 min recorded period was measured in centimeters (cm). Locomotor activity was also further analyzed in 150 min following the removal of the first 30 min of activity deemed as the habituation period. Both measurements of locomotor activity were analyzed by means of a one-way ANOVA followed by a Tukey's post-hoc test to determine significance between treatment groups (Dyck et al., 2012b; Dyck et al., 2011).

3.9.2. Social Interaction Analysis

Analyzed parameters of (1) total amount of time spent interacting, (2) total number of interactions, (3) total number of active interactions, and (4) total number of passive interactions were recorded. Any interaction during the first 5 minutes was excluded as habituation and only interactions made during the last 5 minutes of the recording were deemed as actual activity. Parameters were analyzed by means of a one-way ANOVA followed by a Tukey's post-hoc test to determine significance between treatment groups (Dyck et al., 2012b; Dyck et al., 2011).

3.9.3. Prepulse Inhibition Analysis

Prepulse inhibition was performed according to the procedure previously described by Tenn *et al.* and Dyck *et al.* (Tenn et al., 2003; Dyck et al., 2011; Dyck et al., 2012b; Tenn et al., 2005). The following formula calculates prepulse inhibition output: %PPI = 100 - (P+S/S) * 100. In this equation, P+S is the mean response amplitude for

prepulse-startle pulse trials, and S is the mean response amplitude for startle-only pulse trials. % PPI obtained from this equation was obtained between treatment groups and analyzed by means of a one-way ANOVA followed by a Tukey's post-hoc test to determine significance between treatment groups, at the presentation of the varying prepulses (i.e. 68dB, 71dB, 77dB) (Dyck et al., 2012b; Dyck et al., 2011).

3.9.4. Immunoblotting Analysis

Total area of protein bands for the specific bands of interest obtained from NIH ImageJ program was analyzed by means of a one-way ANOVA followed by a Tukey's post-hoc test to determine significance between treatment groups. This analysis was done individually for synapsin isoforms IIa and IIb, as well vesicular transporters and enzymes associated with dopamine, glutamate, and GABA neurotransmission (Dyck et al., 2011).

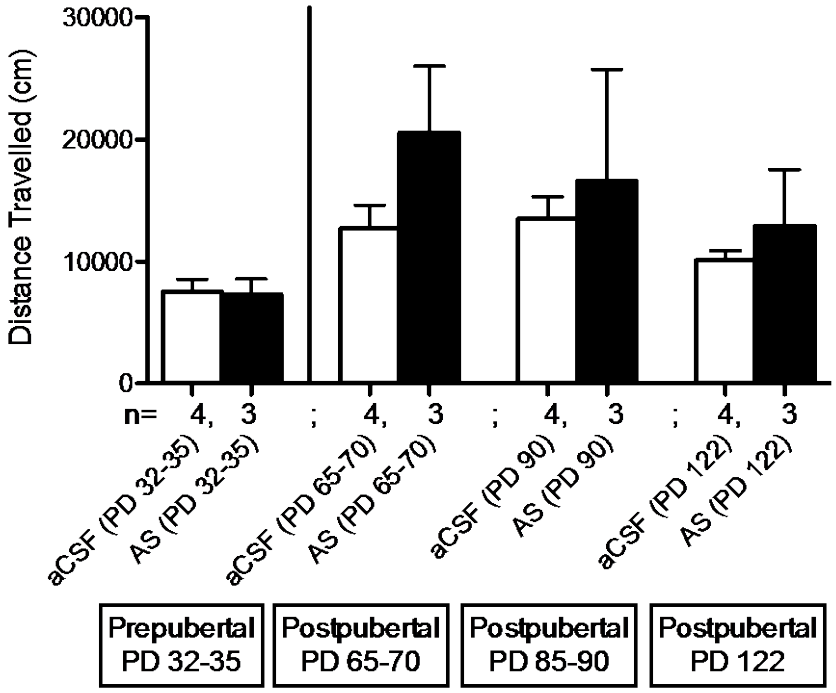
4. RESULTS

4.1. Effect of PD 7 synapsin II knockdown on the development of schizophrenia-like behavioural abnormalities

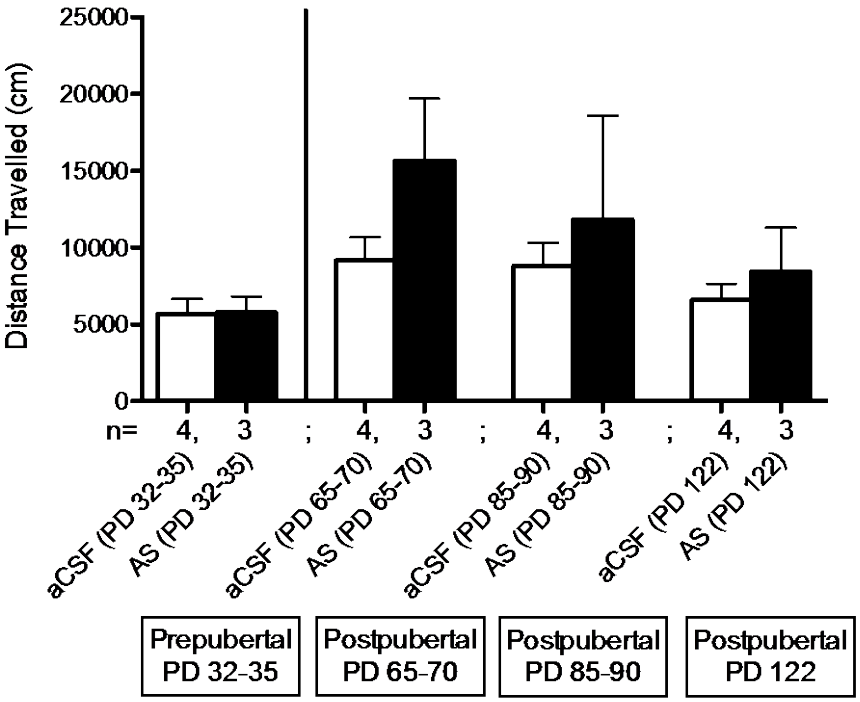
4.1.1 Effect of PD 7 synapsin II knockdown on locomotor activity

A one-time synapsin II knockdown at PD 7 did not induce locomotor changes at both pre-pubertal and post-pubertal stages of adulthood. No significant increase in locomotor activity was found in synapsin II knockdown (AS-treated) animals when compared to aCSF-treated control animals at both pre-pubertal (PD 32-35) and post-pubertal (PD 65-79, PD 85-90, PD 168-169) stages. This insignificant trend was observed in both 180 minutes (**Figure 10A**) and 150 minutes (**Figure 10B**) of locomotor activity recording.

(A) Locomotor activity in 180 mins as measured over lifetime



(B) Locomotor activity in 150 mins as measured over lifetime

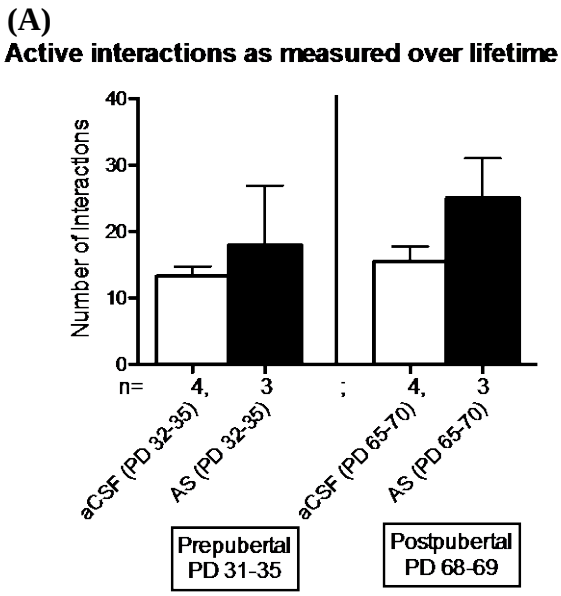


Figures 10A-B: Locomotor activity was measured at both pre-pubertal (PD 31-35) and post-pubertal (PD 65-70, PD 85-90, PD 122) stages. Following a one-time synapsin II knockdown (PD 7), no significant increase in locomotor activity was found in synapsin II knockdown (AS-treated) animals when compared to aCSF-treated control animals at both pre-pubertal and post-pubertal stages. This same trend was observed at both (**A**) 150 minutes and (**B**) 180 minutes of recording. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars and AS-treated group is indicated by black bar.

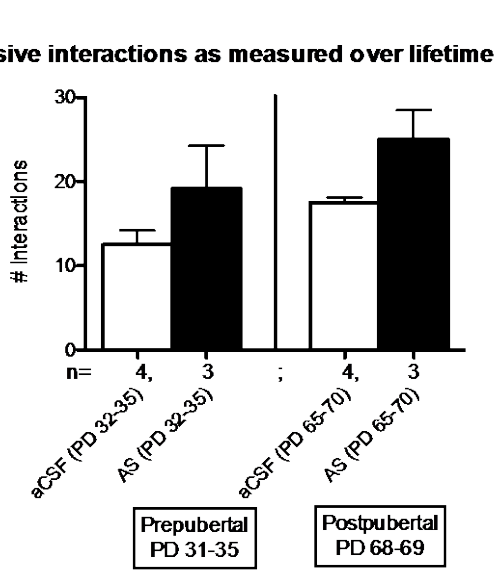
4.1.2 Effect of PD 7 synapsin II knockdown on social interaction

A one-time synapsin II knockdown at PD 7 did not induce changes in number of active interactions, number of passive interactions, and total number of interactions made at both pre-pubertal and post-pubertal stages of adulthood (**Figures 11A-C**). A significant decrease in total time interacted was found in aCSF-treated animals as compared to AS-treated animals: $t(8) = 3.601$, $^{**}p < 0.01$ at post-pubertal stage PD 65-70 (**Figure 11D**).

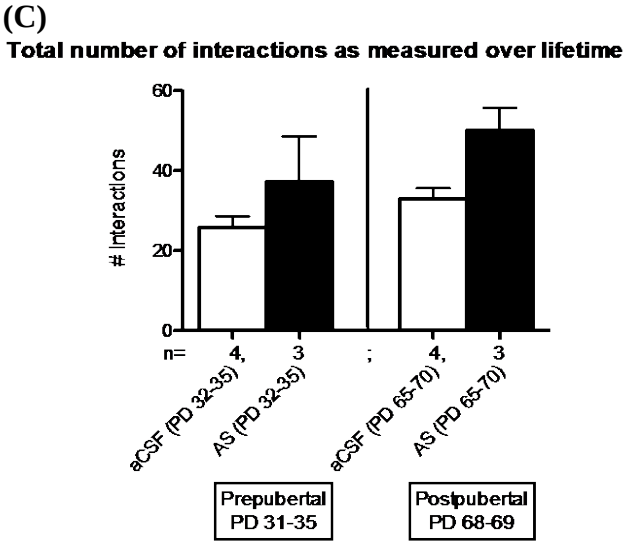
(A)


Active interactions as measured over lifetime
Number of Interactions
40
30
20
10
0
n= 4, 3 ; 4, 3
aCSF (PD 32-35)
AS (PD 32-35)
aCSF (PD 65-70)
AS (PD 65-70)
Prepubertal PD 31-35
Postpubertal PD 68-69

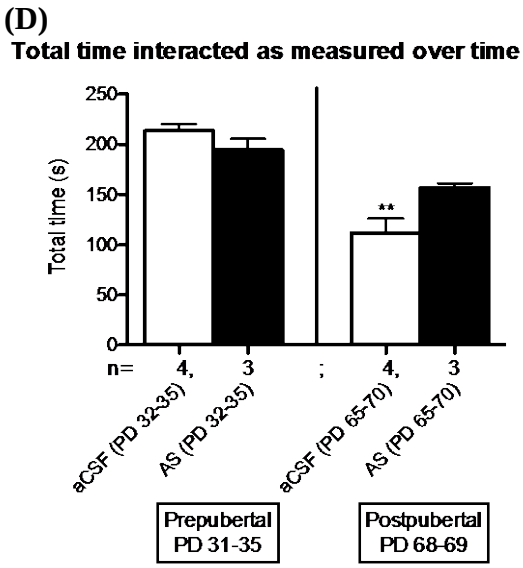
(B)


Passive interactions as measured over lifetime
Interactions
30
20
10
0
n= 4, 3 ; 4, 3
aCSF (PD 32-35)
AS (PD 32-35)
aCSF (PD 65-70)
AS (PD 65-70)
Prepubertal PD 31-35
Postpubertal PD 68-69

(C)


Total number of interactions as measured over lifetime
Interactions
60
40
20
0
n= 4, 3 ; 4, 3
aCSF (PD 32-35)
AS (PD 32-35)
aCSF (PD 65-70)
AS (PD 65-70)
Prepubertal PD 31-35
Postpubertal PD 68-69

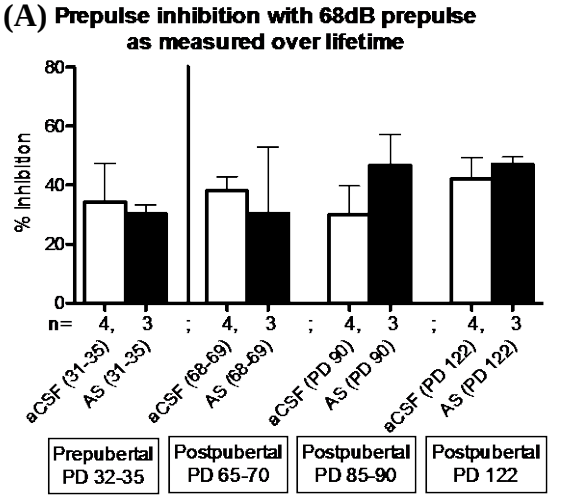
(D)

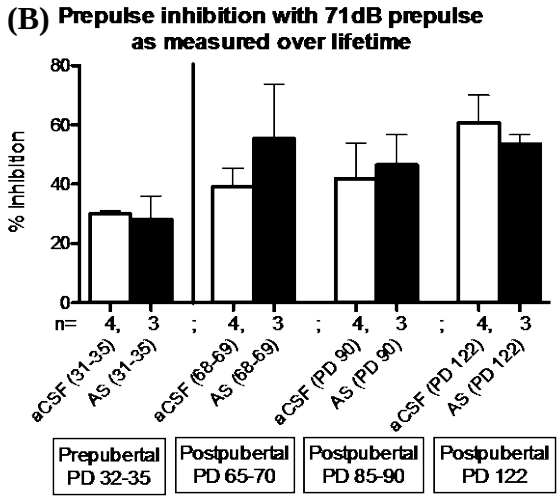

Total time interacted as measured over time
Total time (s)
250
200
150
100
50
0
**
n= 4, 3 ; 4, 3
aCSF (PD 32-35)
AS (PD 32-35)
aCSF (PD 65-70)
AS (PD 65-70)
Prepubertal PD 31-35
Postpubertal PD 68-69

Figures 11A-D: Social interaction was measured at both pre-pubertal (PD 31-35) and post-pubertal (PD 65-70) stages. Parameters measured include number of active interactions, number of passive interactions, total number of interactions, and total time interacted (seconds). Following a one-time synapsin II knockdown (PD 7), no significant decrease in social interaction was found in synapsin II knockdown (AS-treated) animals when compared to aCSF-treated control animals in parameters of (**A**)active interactions, (**B**) passive interactions, and (**C**) total number of interactions at both pre-pubertal and post-pubertal stages. (**D**) A decrease in time spent interacting was found in aCSF treatment as compared to AS treatment at post-pubertal stage PD 65-70, $t(8) = 3.601$, $^{**}p < 0.01$. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars and AS-treated group is indicated by black bar. $^{**}p < 0.01$

4.1.3 Effect of PD 7 synapsin II knockdown on prepulse inhibition

A one-time synapsin II knockdown at PD 7 did not induce deficits in prepulse inhibition, at 68dB, 71dB, and 77dB of prepulse decibels (**Figures 12A-C**). Prepulse inhibition deficits were not detected at both pre-pubertal (PD 32-35) and post-pubertal (PD 65-70, PD 85-90, PD 122) stages in AS-treated animals as when compared to aCSF-treated controls.


(A) Prepulse inhibition with 68dB prepulse as measured over lifetime
% Inhibition
80
60
40
20
0
n= 4, 3 ; 4, 3 ; 4, 3 ; 4, 3
aCSF (31-35)
AS (31-35)
aCSF (68-69)
AS (68-69)
aCSF (PD 90)
AS (PD 90)
aCSF (PD 122)
AS (PD 122)
Prepubertal PD 32-35
Postpubertal PD 65-70
Postpubertal PD 85-90
Postpubertal PD 122


(B) Prepulse inhibition with 71dB prepulse as measured over lifetime
% Inhibition
80
60
40
20
0
n= 4, 3 ; 4, 3 ; 4, 3 ; 4, 3
aCSF (31-35)
AS (31-35)
aCSF (68-69)
AS (68-69)
aCSF (PD 90)
AS (PD 90)
aCSF (PD 122)
AS (PD 122)
Prepubertal PD 32-35
Postpubertal PD 65-70
Postpubertal PD 85-90
Postpubertal PD 122

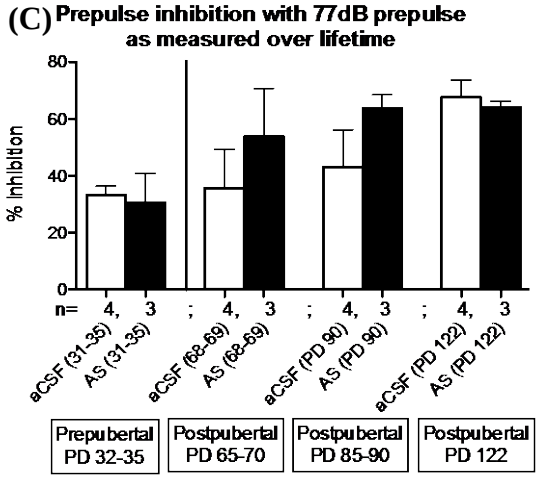

(C) Prepulse inhibition with 77dB prepulse as measured over lifetime
% Inhibition
80
60
40
20
0
n= 4, 3 ; 4, 3 ; 4, 3 ; 4, 3
aCSF (31-35)
AS (31-35)
aCSF (68-69)
AS (68-69)
aCSF (PD 90)
AS (PD 90)
aCSF (PD 122)
AS (PD 122)
Prepubertal PD 32-35
Postpubertal PD 65-70
Postpubertal PD 85-90
Postpubertal PD 122

Figure 12A-C: Prepulse inhibition was measured at both pre-pubertal (PD 31-35) and post-pubertal (PD 68-69, PD 90, PD 122) stages. Following a one-time synapsin II knockdown (PD 7), no significant deficit in prepulse inhibition was found in synapsin II knockdown (AS-treated) animals when compared to aCSF-treated control animals at both pre-pubertal and post-pubertal stages. This same trend was found with prepulses (**A**) 68dB, (**B**) 71dB, and (**C**) 77dB. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars and AS-treated group is indicated by black bar.

4.2. Efficacy of synapsin II knockdown and purity of olanzapine

4.2.1. Visualization of successful infusion into the medial prefrontal cortex

Prior advancing into behavioural and neurochemical assessments at adulthood, the efficacy and specificity of synapsin II knockdown was first assessed on PD 24 immediately after the final day of infusion. Synapsin II infusion was histologically verified to target the mPFC of the PD 24 neonate (**Figure 13**).

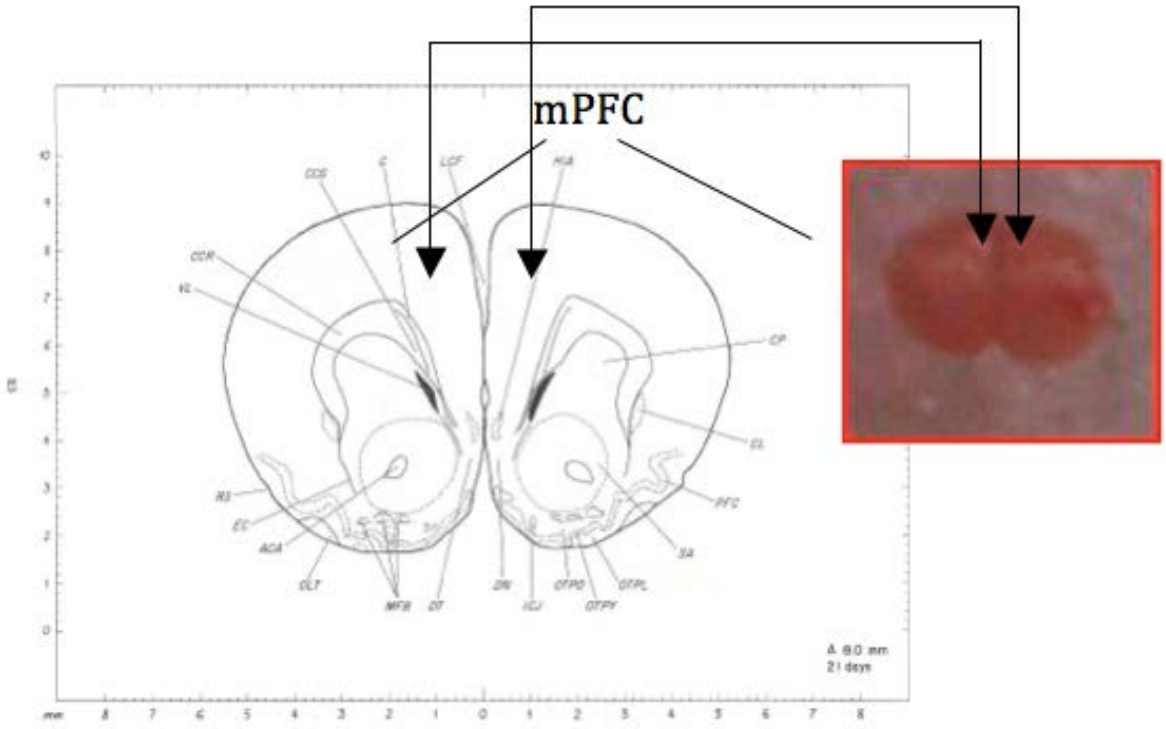


Figure 13: Following 7 consecutive days of infusion (PD 17-23), a random rat was selected and sacrificed at PD 24 to histologically verify the infusion site. Dark dots on the brain slice (as indicated by arrows) point to the mPFC and indicate the specificity of the implanted brain cannulas into the targeted brain region.

4.2.2. Efficacy of synapsin II knockdown

Immunoblotting was performed on PD 24 to verify knockdown of synapsin II in the mPFC after 7 consecutive days of infusion (PD 17-23). Both synapsin IIa and IIb isoforms were significantly reduced in the AS-treated neonate as compared to aCSF-treated and sense-treated control neonate (**Figure 14**). With synapsin IIa protein expression levels: $F(2,7) = 19.62$ in the one-way analysis of variance; aCSF vs. AS ** $p < 0.01$, sense vs. AS ** $p < 0.01$ in post-hoc analyses. With synapsin IIb protein expression levels: $F(2,7) = 8.749$ in the one-way analysis of variance; aCSF vs. AS * $p < 0.05$, sense vs. AS * $p < 0.05$ in post-hoc analyses.

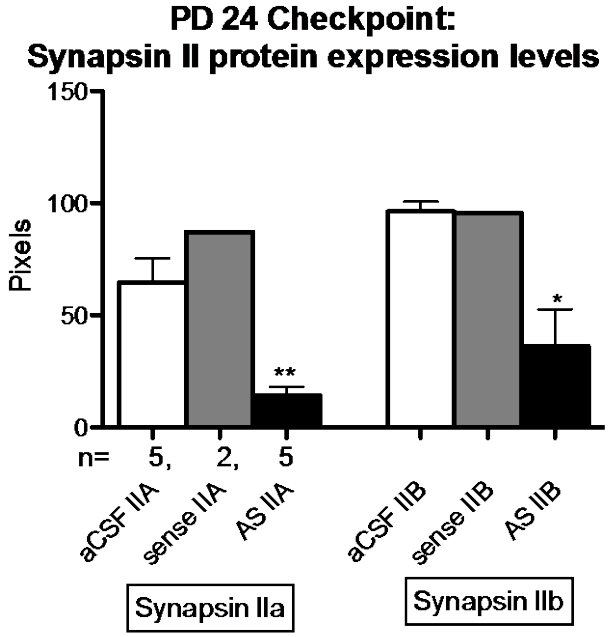
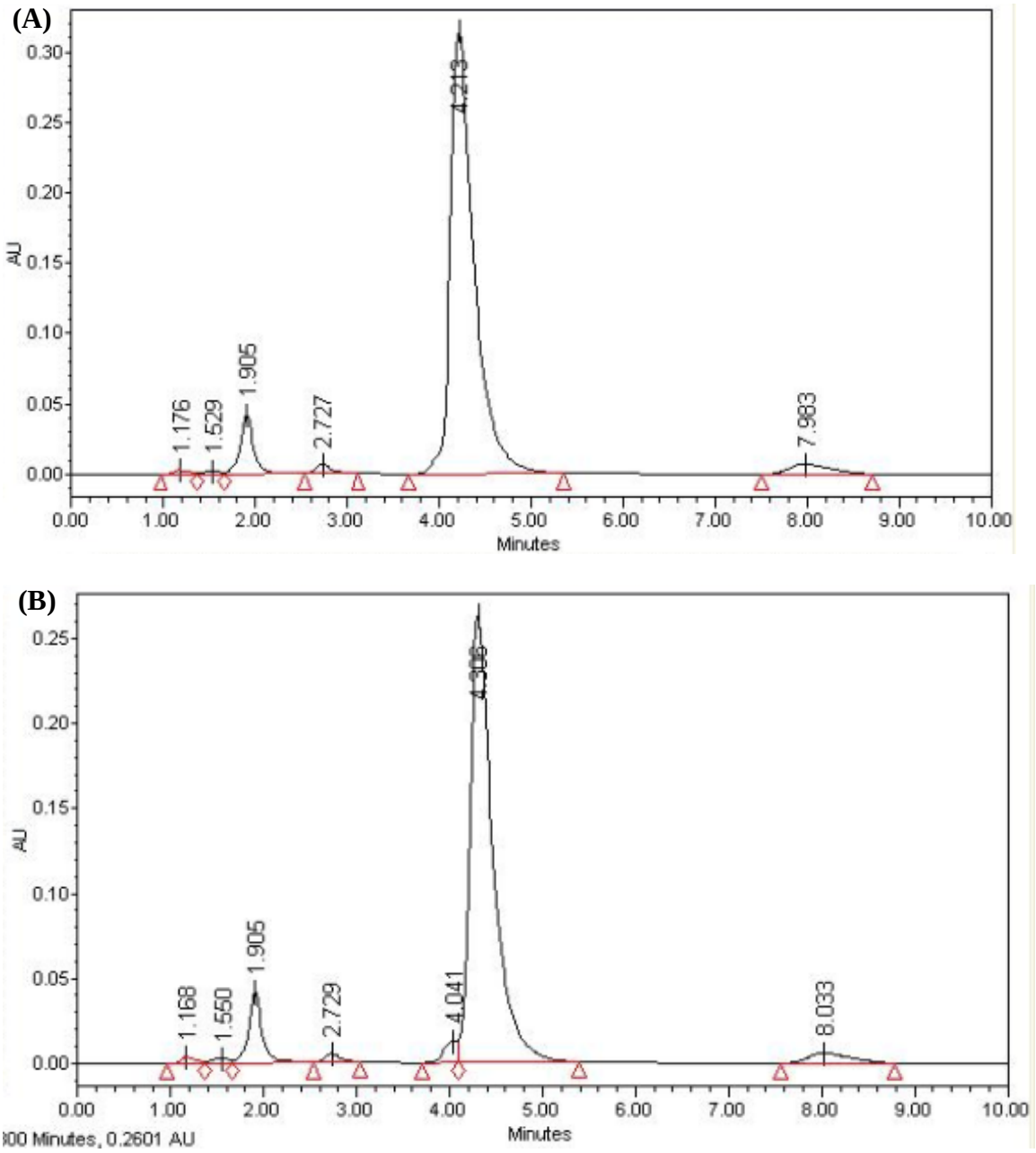


Figure 14: Immunoblotting assessed protein expression levels of synapsin IIa and IIb on PD 24, following a 7-day knockdown (PD 17-23). Both synapsin II isoforms IIa and IIb were significantly reduced with AS treatment. With synapsin IIa protein expression levels: $F(2,7) = 19.62$. With synapsin IIb protein expression levels: $F(2,7) = 8.749$. *$p<0.05$, **$p<0.01$.

4.2.3. Purity of olanzapine

HPLC testing revealed the degree of purity of in-house extracted olanzapine from Eli Lily tablets. **Figure 15** indicate a similar retention time of our extracted olanzapine sample (**B:** 4.213 minutes) as compared to purchased pure olanzapine standard from Sigma (**A:** 4.306 minutes), at a concentration of 0.1mg/ml.



Figures 15A-B: Purity of olanzapine was assessed with HPLC. Our extracted sample (**B**) showed similar retention time of approximately 4 minutes as compared to a purchased pure olanzapine sample (**A**) at select concentration presented here (0.1 mg/ml). Other background peaks were found similar in both extracted sample (**B**) and pure standard (**A**), and could indicate either other metabolites of olanzapine or impurities found in the vehicle solvent.

4.3. Effect of PD 17-23 synapsin II knockdown on the development of schizophrenia-like behavioural abnormalities and the effect of acute olanzapine treatment

4.3.1. Effect of PD 17-23 synapsin II knockdown on locomotor activity

Following 7 consecutive days of neurodevelopmental synapsin II knockdown (PD 17-23), locomotor activity was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages (**Figures 16A-B**). Alterations in locomotor activity were found resulting from the various treatments at both 180 minutes and 150 minutes of recording.

At 180 minutes of locomotor activity recording, synapsin II knockdown rats displayed an increase in locomotor activity at most stages of development as when compared to aCSF-treatment and AS + olanzapine treatment animals. Sense-treatment also caused a significance increase in locomotor activity when compared to aCSF-treated controls. At pre-pubertal stage PD 32-35, locomotor activity was significantly different amongst treatment groups: $F(3,87)$: =19.04 in the one-way analysis of variance; aCSF vs. sense $*p < 0.05$, aCSF vs. AS $***p < 0.001$, and AS vs. AS + olanzapine $***p < 0.001$ in post-hoc analyses. At post-pubertal stage PD 55-59, locomotor activity of aCSF vs. AS was significant as measured by a student's t-test: $t(21) = 3.011$, $p = 0.0067$. At post-pubertal stage PD 65-70, locomotor activity similarly significantly different amongst treatment groups: $F(3,61) = 6.041$ in the one-way analysis of variance; aCSF vs. sense $**p < 0.01$, aCSF vs. AS $*p < 0.05$, sense vs. AS + olanzapine $*p < 0.05$ in post-hoc analyses. At post-pubertal stage PD 168-169, locomotor activity of aCSF vs. AS was

significantly different as measured by a student's t-test: t(9) = 2.845, p = 0.0192 (**Figure 16A**).

With the removal of the initial 30 minutes of habituation, 150 minutes of locomotor activity recording indicated that synapsin II knockdown rats displayed an increase in locomotor activity both pre-pubertal stage PD 32-35 and post-pubertal stage PD 55-59 as when compared to aCSF-treatment, and AS + olanzapine treatment animals. At pre-pubertal stage PD 32-35, locomotor activity were significantly different amongst treatment groups: F(3,86): =12.69 in the one-way analysis of variance; aCSF vs. AS ***p < 0.001, and AS vs. AS + olanzapine *p < 0.05 in post-hoc analyses. At post-pubertal stage PD 55-59, locomotor activity of aCSF vs. AS was significantly different as measured by a student's t-test: t(21) = 3.310, p = 0.0033 (**Figure 16B**).

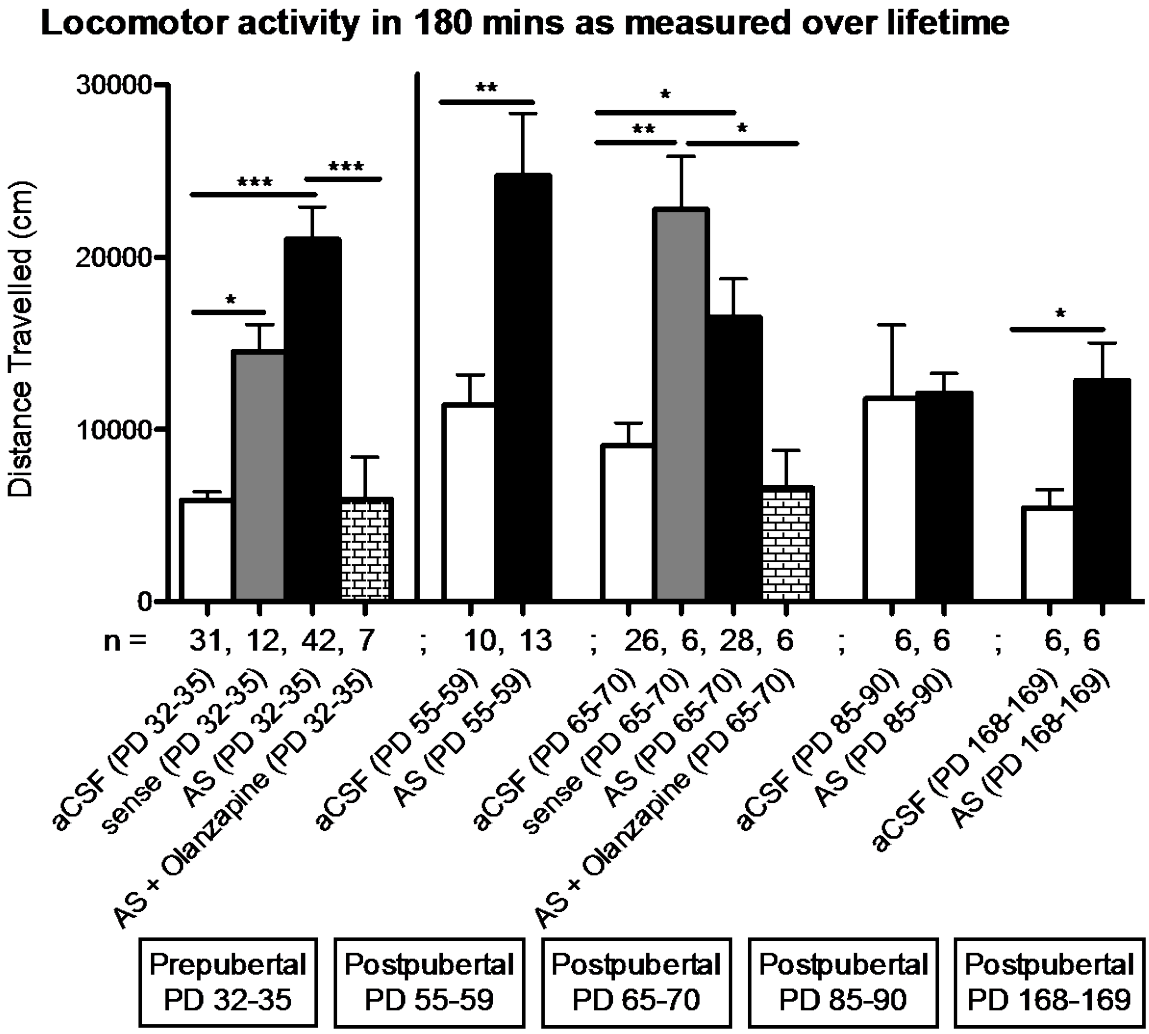


Figure 16A: Following developmental synapsin II knockdown (PD 17-23), locomotor activity was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages. Graph indicates total distance travelled as measured in centimeters (cm) over a 180 minute recording period in aCSF-, sense-, AS-, and AS + olanzapine- treated animals. Synapsin II knockdown (AS-treated) animals were found to be significantly more active when compared to aCSF-treated control animals. A significant increase in locomotor activity was measured at pre-pubertal stages and persisted into post-pubertal stages. Treatment with antipsychotic drug, olanzapine, was

found to significantly attenuate the increase in locomotor activity. Sample size within each treatment group is indicated below the x-axis of the graph. At PD 32-35, $F(3,87)$: =19.04, *$p < 0.05$, ***$p < 0.001$. At PD 55-59, $t(21) = 3.011$, $p = 0.0067$, **$p < 0.01$. At PD 65-70, $F(3,61)=6.041$, *$p < 0.05$, **$p < 0.01$. At PD 168-169, $t(9) = 2.845$, $p = 0.0192$, *$p < 0.05$. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. *$p<0.05$; **$p<0.01$; ***$p<0.001$.

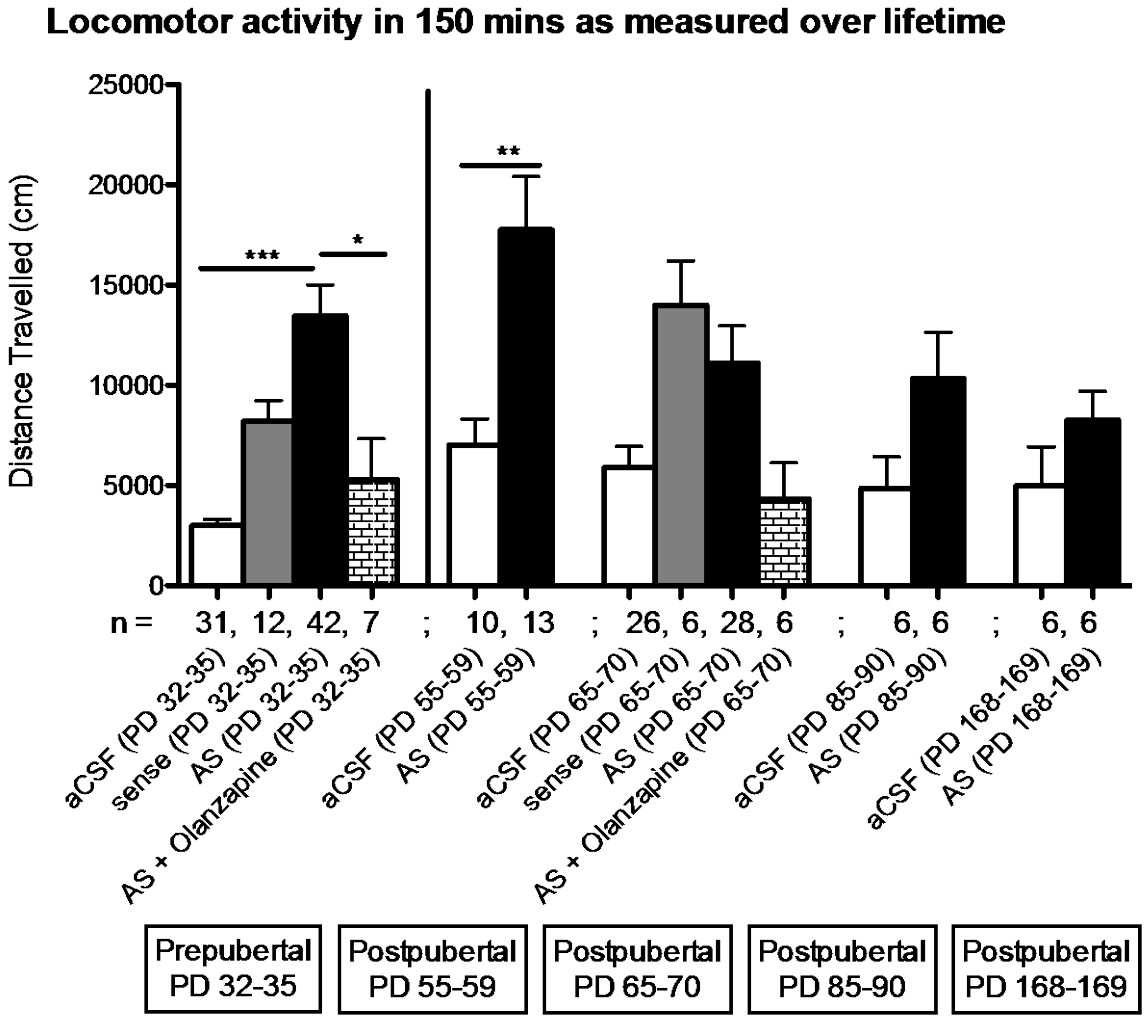


Figure 16B: Following developmental synapsin II knockdown (PD 17-23), locomotor activity was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages. Graph indicates total distance travelled as measured in centimeters (cm) over a 150 minute recording period in aCSF-, sense-, AS- , and AS + olanzapine- treated animals. Synapsin II knockdown (AS-treated) animals were found to be significantly more active when compared to aCSF-treated control animals. A significant increase in locomotor activity was measured at pre-pubertal stages and persisted into post-pubertal stages. Treatment with antipsychotic drug, olanzapine, was

found to significantly attenuate the increase in locomotor activity. Sample size within each treatment group is indicated below the x-axis of the graph. At PD 32-35, $F(3,86)$: $=12.69$, $*p < 0.05$, $***p < 0.001$. At PD 55-59, $t(21) = 3.310$, $p = 0.0033$, $**p < 0.01$. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. $*p<0.05$; $**p<0.01$; $***p<0.001$.

4.3.1.1. Effect of acute antipsychotic drug olanzapine treatment on locomotor activity

AS-treatment caused a 256% increase in locomotor activity at pre-pubertal stage PD 32-35, a 116% increase at post-pubertal stage PD 55-59, and a 82% increase at post-pubertal stage PD 65-70 as compared to aCSF-treated control animals (**$p < 0.01$, ***$p < 0.0001$). Olanzapine treatment effectively attenuated this increase in locomotor activity by 72% at pre-pubertal stage PD 32-35 and by 60% at post-pubertal stage PD 65-70 in AS + olanzapine treated animals as when compared to AS-treated animals (*$p < 0.05$) (**Figures 16A-B**).

4.3.1.2 Effect of gender on locomotor activity

In order to identify the effects of the gender differences of locomotor activity resulting from neurodevelopmental synapsin II knockdown, locomotor activity was analyzed in the male and female rats separately. Locomotor activity was reported at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages (**Figures 17A-B, 18A-B, 19A-B**). A significant difference in locomotor activity was found in both male and female populations when examining the differences between treatment groups both at 180 minutes and 150 minutes of recording. Both populations, however, show differing degrees of robustness at different stages of development.

At 180 minutes of locomotor activity recording in the male population, synapsin II knockdown rats displayed an increase in locomotor activity at most stages of development as when compared to aCSF-treatment, and AS + olanzapine treatment

animals. Sense-treatment also caused a significance increase in locomotor activity when compared to aCSF-treated controls. At pre-pubertal stage PD 32-35, locomotor activity was significantly different amongst treatment groups: $F(3,48)$: =11.00 in the one-way analysis of variance; aCSF vs. sense $*p < 0.05$, aCSF vs. AS $***p < 0.001$, and AS vs. AS + olanzapine $**p < 0.001$ in post-hoc analyses. At post-pubertal stage PD 65-70, locomotor activity during was similarly significantly different amongst treatment groups: $F(3,32) = 4.673$ in the one-way analysis of variance; aCSF vs. sense $**p < 0.01$. At post-pubertal stage PD 168-169, locomotor activity of aCSF vs. AS was significantly different as measured by a student's t-test: $t(5) = 2.659$, $p = 0.0450$ (**Figure 17A**).

With the removal of the initial 30 minutes of habituation, 150 minutes of locomotor activity recording in the male population indicated that synapsin II knockdown rats displayed an increase in locomotor activity at prepubertal stage PD 32-35, postpubertal stage PD 65-70, and post-pubertal stage PD 168-169 as when compared to aCSF-treatment, and AS + olanzapine treatment animals. At prepubertal stage PD 32-35, locomotor activity were significantly different amongst treatment groups: $F(3,46)$: = 9.215 in the one-way analysis of variance; aCSF vs. AS $***p < 0.001$, and AS vs. AS + Olanzapine $*p < 0.05$ in post-hoc analyses. At post-pubertal stage PD 55-59, locomotor activity of aCSF vs. AS was significant during as measured by a student's t-test: $t(12) = 2.268$, $p = 0.0426$. At post-pubertal stage PD 168-169, locomotor activity of aCSF vs. AS was significantly different as measured by a student's t-test: $t(5) = 2.664$, $p = 0.0446$. (**Figure 17B**).

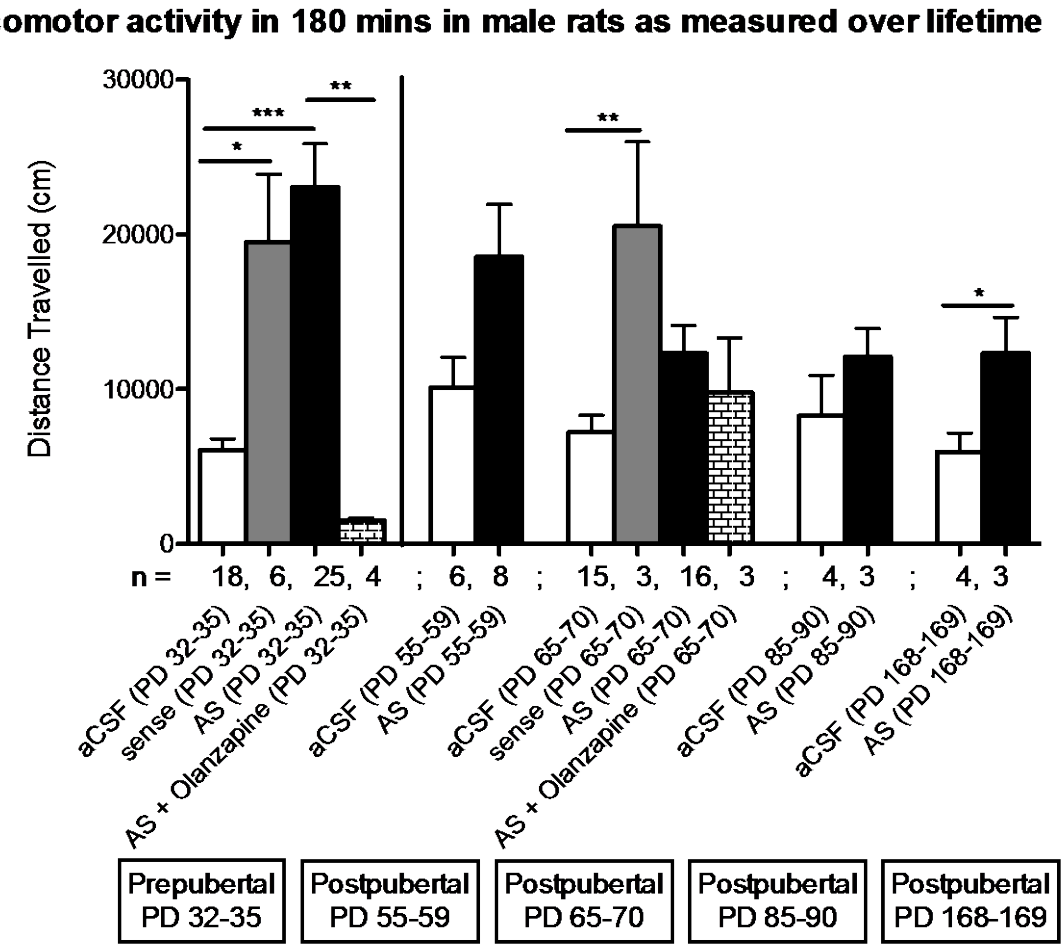


Figure 17A: Following developmental synapsin II knockdown (PD 17-23), locomotor activity was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages in male animals. Graph indicates total distance travelled as measured in centimeters (cm) over a 180 minute recording period in aCSF-, sense-, AS-, and AS + olanzapine- treated male animals. Synapsin II knockdown (AS-treated) male animals were found to be significantly more active when compared to aCSF-treated control animals at select stages. A significant increase in locomotor activity was measured at pre-pubertal stages and persisted into post-pubertal stages. Treatment with antipsychotic drug, olanzapine, was found to attenuate the increase in locomotor activity. Sample size within each treatment group is indicated below the x-axis of the

graph. At PD 32-35, F(3,48): =11.00, *p < 0.05, **p < 0.01, ***p < 0.001. At PD 55-59, t(21) = 3.011, p = 0.0067, **p < 0.01. At PD 65-70, F(3,32) = 4.673, **p < 0.01. At PD 168-169t(5) = 2.659, p = 0.0450, *p < 0.05. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. *p<0.05; **p<0.01; ***p<0.001.

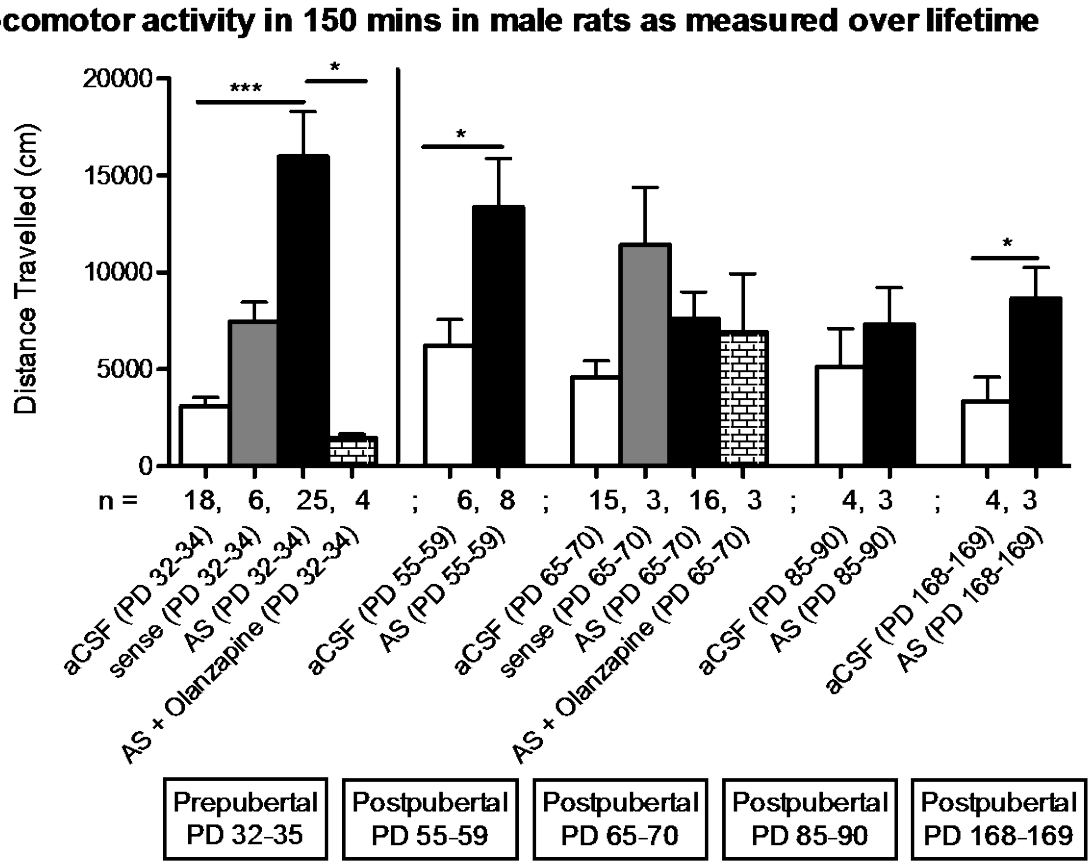


Figure 17B: Following developmental synapsin II knockdown (PD 17-23), locomotor activity was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages in male animals. Graph indicates total distance travelled as measured in centimeters (cm) over a 150 minute recording period in aCSF-, sense-, AS-, and AS + olanzapine- treated male animals. Synapsin II knockdown (AS-treated) animals were found to be significantly more active when compared to aCSF-treated control animals at select stages. A significant increase in locomotor activity was measured at pre-pubertal stages and persisted into post-pubertal stages. Treatment with antipsychotic drug, olanzapine, was found to significantly attenuate the increase in locomotor activity. Sample size within each treatment group is indicated below the x-axis of the graph. At PD 32-35, $F(3,46): = 9.215$, $*p < 0.05$, $***p < 0.001$. At PD 55-59, $t(12)$

= 2.268, p = 0.0426, *p < 0.05. At PD 168-169, t(5) = 2.664, p = 0.0446, *p < 0.05. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. *p<0.05; ***p<0.001.

At 180 minutes of locomotor activity recording in the female population, synapsin II knockdown rats displayed an increase in locomotor activity at pre-pubertal stage PD 32-35 and and post-pubertal stage PD 55-59 as when compared to aCSF-treatment. At pre-pubertal stage PD 32-35, locomotor activity was significantly different amongst treatment groups: $F(3,35): = 8.306$ in the one-way analysis of variance; aCSF vs. AS ***$p < 0.001$ in post-hoc analyses. At post-pubertal stage PD 55-59, locomotor activity of aCSF vs. AS was significantly different as measured by a student's t-test: $t(6) = 2.455$, $p = 0.0495$ (**Figure 18A**).

At 150 minutes of locomotor activity recording in the female population, synapsin II knockdown rats displayed a similar increase in locomotor activity at pre-pubertal stage PD 32-35 and post-pubertal stage PD 55-59 as when compared to aCSF-treatment. At prepubertal stage PD 32-35, locomotor activity were significantly different amongst treatment groups: $F(3,35): = 5.537$ in the one-way analysis of variance; aCSF vs. AS **$p < 0.01$ in post-hoc analyses. At post-pubertal stage PD 55-59, locomotor activity of aCSF vs. AS was significantly different as measured by a student's t-test: $t(7) = 3.163$, $p = 0.0159$ (**Figure 18B**).

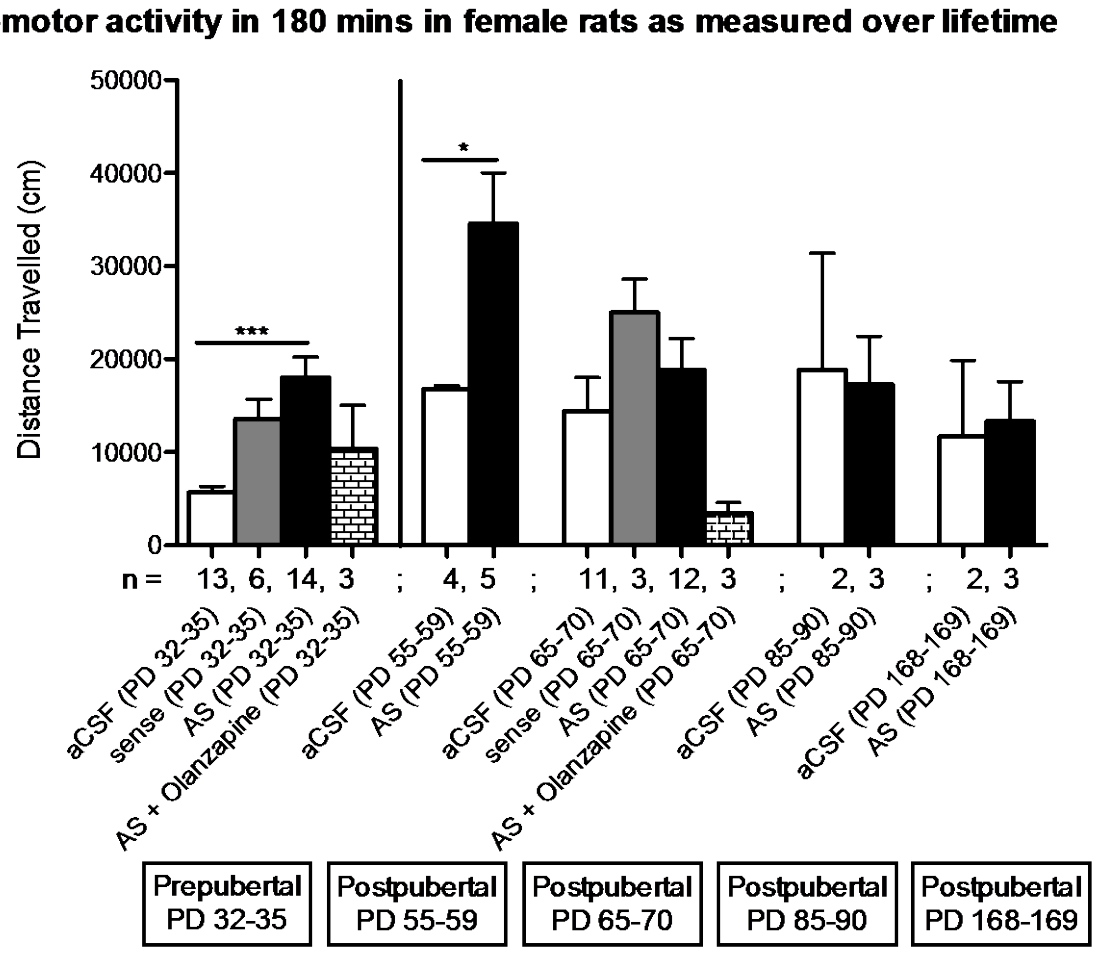


Figure 18A: Following developmental synapsin II knockdown (PD 17-23), locomotor activity was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages in female animals. Graph indicates total distance travelled as measured in centimeters (cm) over a 180 minute recording period in aCSF-, sense-, AS-, and AS + olanzapine- treated female animals. Synapsin II knockdown (AS-treated) female animals were found to be significantly more active when compared to aCSF-treated control animals at select stages. A significant increase in locomotor activity was measured at pre-pubertal stages and persisted into post-pubertal stages. Treatment with antipsychotic drug, olanzapine, was found to attenuate the increase in locomotor activity. Sample size within each treatment group is indicated below the x-axis of the

graph. At PD 32-35, $F(3,35): = 8.306$, ***$p < 0.001$. At PD 55-59, $t(6) = 2.455$, $p = 0.0495$, *$p < 0.05$.Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. *$p<0.05$; ***$p<0.001$.

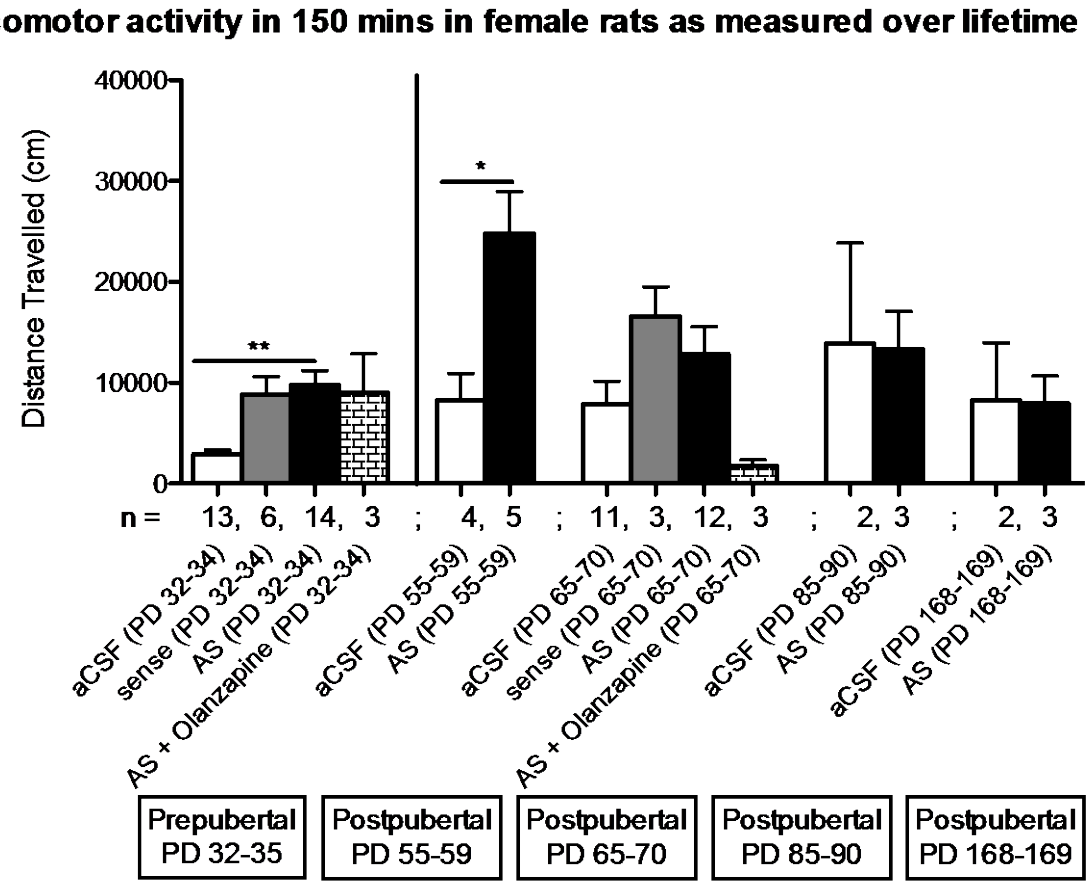


Figure 18B: Following developmental synapsin II knockdown (PD 17-23), locomotor activity was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages in female animals. Graph indicates total distance travelled as measured in centimeters (cm) over a 150 minute recording period in aCSF-, sense-, AS-, and AS + olanzapine- treated female animals. Synapsin II knockdown (AS-treated) animals were found to be significantly more active when compared to aCSF-treated control animals at select stages. A significant increase in locomotor activity was measured at pre-pubertal stages and persisted into post-pubertal stages. Treatment with antipsychotic drug, olanzapine, was found to attenuate the increase in locomotor activity. Sample size within each treatment group is indicated below the x-axis of the graph. At PD 32-35, $F(3,35)$: = 5.537, $^{**}p < 0.01$. At PD 55-59, $t(7) = 3.163$, $p = 0.0159$, $^{*}p < 0.05$.

Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. *$p<0.05$; **$p<0.01$.

At the 180 minutes of recording, few gender differences in locomotor activity were observed in the various treatment groups. At post-pubertal stage PD 55-59, locomotor activity of AS-treated male vs. female was significantly different as measured by a student's t-test: $t(11) = 2.651$, $p = 0.0226$. At post-pubertal stage PD 65-70, locomotor activity of aCSF-treated male vs. female was significantly different as measured by a student's t-test: $t(2) = 2.186$, $p = 0.0388$ (**Figure 19A**).

At the 150 minutes of recording, few gender differences in locomotor activity were observed as a result of synapsin II neurodevelopmental knockdown. At pre-pubertal stage PD 32-35, locomotor activity of AS-treated male vs. female was significantly different as measured by a student's t-test: $t(40) = 2.042$, $p = 0.0478$. At post-pubertal stage PD 55-59, locomotor activity of AS-treated male vs. female was significantly different as measured by a student's t-test: $t(11) = 2.528$, $p = 0.0281$ (**Figure 19B**).

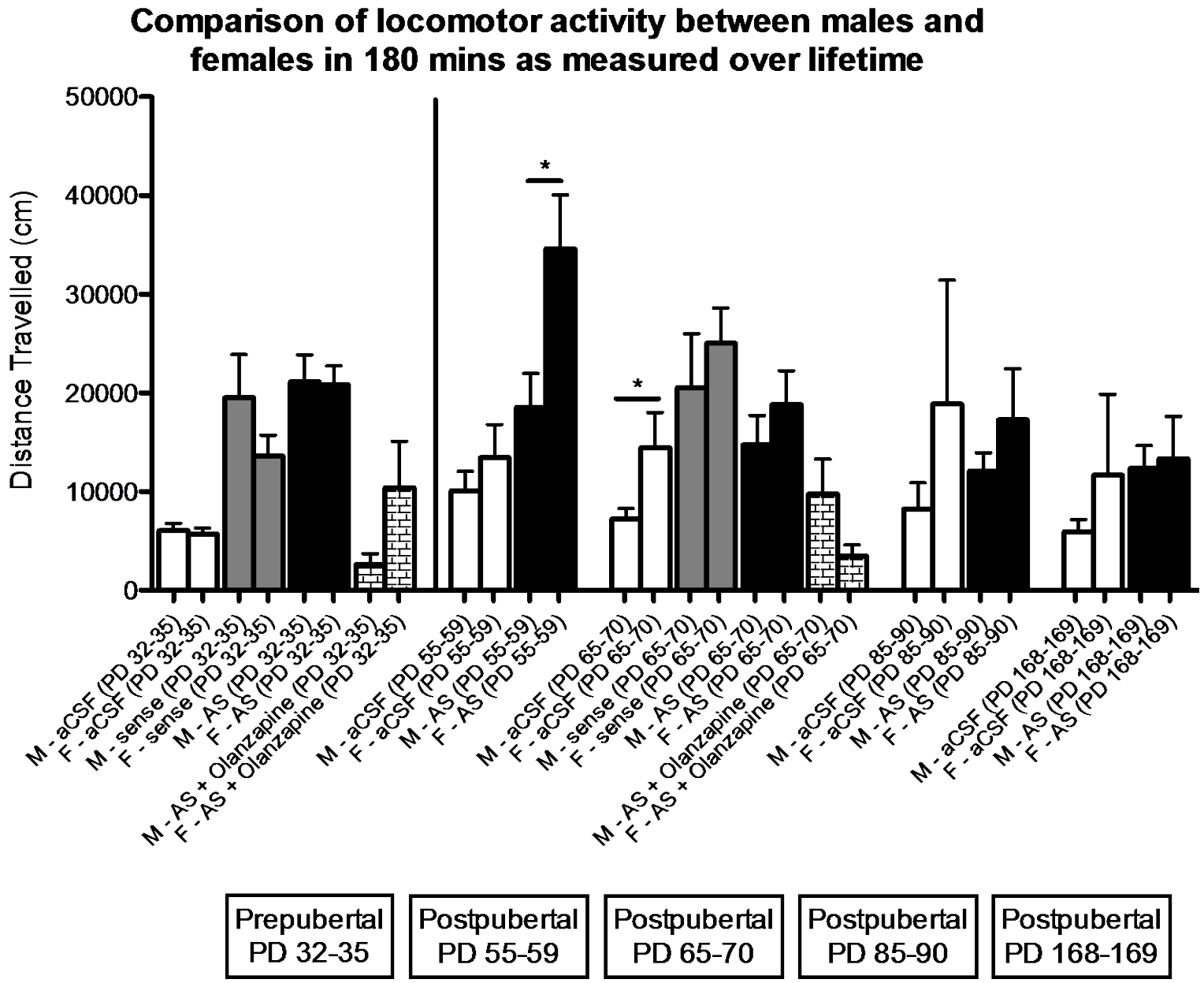

Comparison of locomotor activity between males and females in 180 mins as measured over lifetime
Distance Travelled (cm)
50000
40000
30000
20000
10000
0
*
*
M - aCSF (PD 32-35)
F - aCSF (PD 32-35)
M - sense (PD 32-35)
F - sense (PD 32-35)
M - AS (PD 32-35)
F - AS (PD 32-35)
M - AS + Olanzapine (PD 32-35)
F - AS + Olanzapine (PD 32-35)
M - aCSF (PD 55-59)
F - aCSF (PD 55-59)
M - AS (PD 55-59)
F - AS (PD 55-59)
M - aCSF (PD 65-70)
F - aCSF (PD 65-70)
M - sense (PD 65-70)
F - sense (PD 65-70)
M - AS (PD 65-70)
F - AS (PD 65-70)
M - AS + Olanzapine (PD 65-70)
F - AS + Olanzapine (PD 65-70)
M - aCSF (PD 85-90)
F - aCSF (PD 85-90)
M - AS (PD 85-90)
F - AS (PD 85-90)
M - aCSF (PD 168-169)
F - aCSF (PD 168-169)
M - AS (PD 168-169)
F - AS (PD 168-169)
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70
Postpubertal PD 85-90
Postpubertal PD 168-169

Figure 19A: An analysis of gender differences between the various treatment groups was considered, as a function of the developmental timeline. Locomotor activity was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages, and compared between male and female animals. Graph indicates total distance travelled as measured in centimeters (cm) over a 180 minute recording period in aCSF-, sense-, AS-, and AS+olanzapine- treated female animals. Female (F) animals showed an increase in locomotor activity as compared to male (M) animals with AS treatment at post-pubertal stage PD 55-59 and aCSF treatment at post-pubertal stage PD 65-70. At PD 55-59, $t(11) = 2.651$, $p = 0.0226$, $*p < 0.05$. At PD 65-70, $t(2) = 2.186$, $p = 0.0388$, $*p < 0.05$. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. $*p<0.05$.

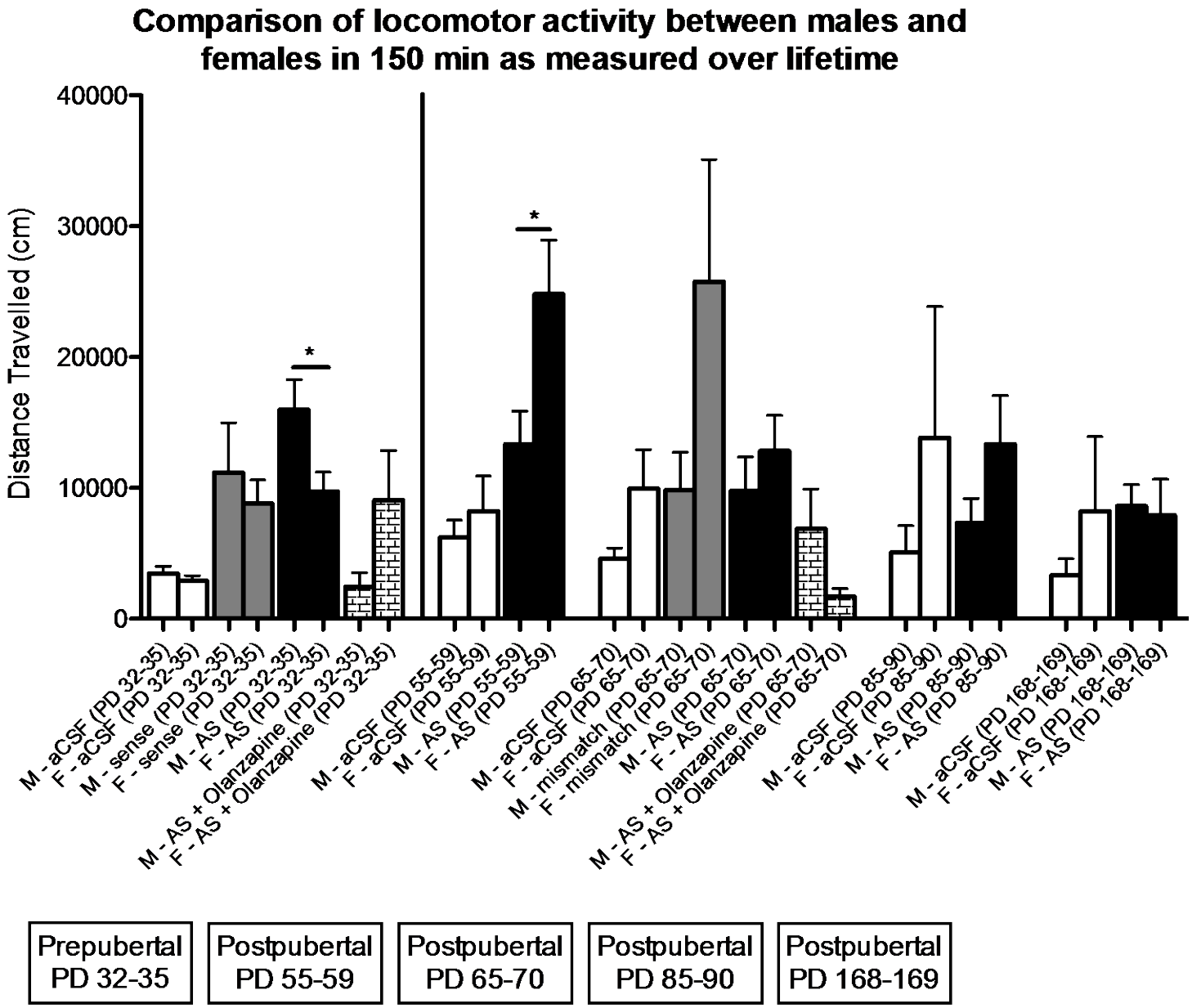

Comparison of locomotor activity between males and females in 150 min as measured over lifetime
Distance Travelled (cm)
40000
30000
20000
10000
0
*
*
M - aCSF (PD 32-35)
F - aCSF (PD 32-35)
M - sense (PD 32-35)
F - sense (PD 32-35)
M - AS (PD 32-35)
F - AS (PD 32-35)
M - AS + Olanzapine (PD 32-35)
F - AS + Olanzapine (PD 32-35)
M - aCSF (PD 55-59)
F - aCSF (PD 55-59)
M - AS (PD 55-59)
F - AS (PD 55-59)
M - aCSF (PD 65-70)
F - aCSF (PD 65-70)
M - mismatch (PD 65-70)
F - mismatch (PD 65-70)
M - AS (PD 65-70)
F - AS (PD 65-70)
M - AS + Olanzapine (PD 65-70)
F - AS + Olanzapine (PD 65-70)
M - aCSF (PD 85-90)
F - aCSF (PD 85-90)
M - AS (PD 85-90)
F - AS (PD 85-90)
M - aCSF (PD 168-169)
F - aCSF (PD 168-169)
M - AS (PD 168-169)
F - AS (PD 168-169)
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70
Postpubertal PD 85-90
Postpubertal PD 168-169

Figure 19B: An analysis of gender differences between the various treatment groups was considered, as a function of the developmental timeline. Locomotor activity was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages, and compared between male and female animals. Graph indicates total distance travelled as measured in centimeters (cm) over a 150 minute recording period in aCSF-, sense-, AS-, and AS+olanzapine- treated female animals. Female (F) animals showed adecrease in locomotor activity with AS treatment at pre-pubertal stage PD 32-35 and an increase in locomotor activity with aCSF treatment at post-pubertal stage PD 55-59, as compared to their similarly treated male (M) animals. At PD 32-35, $t(40) = 2.042$, $p = 0.0478$, $*p < 0.05$. At PD 55-59, $t(11) = 2.528$, $p = 0.0281$, $*p < 0.05$. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. $*p<0.05$.

4.3.2. Effect of PD 17-23 synapsin II knockdown on social interaction

Following 7 consecutive days of neurodevelopmental synapsin II knockdown (PD 17-23) Social interaction was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70) stages (**Figures 20A-D**). Alterations in the various parameters of social interaction (i.e. number of active interactions, number of passive interactions, total number of interactions, and total time interacted) were found resulting from the various treatments (**Figure 20A-D**).

When measuring the number of active interactions, synapsin II knockdown AS-treated rats displayed a decrease in social interaction as when compared to sense-treated and AS + olanzapine- treated animals at pre-pubertal stage PD 32-35 and post-pubertal stage PD 65-70. A decrease in social interaction was found in aCSF-treated animals when compared to sense-treated and AS + olanzapine- treated animals at pre-pubertal stage PD 32-35. At pre-pubertal stage PD 32-35, social interaction was significantly different amongst treatment groups: $F(3,99)$: =16.18 in the one-way analysis of variance; aCSF vs. sense $^{***}p < 0.001$, aCSF vs. AS + olanzapine $^{**}p < 0.01$, sense vs. AS $^{***}p < 0.001$, and AS vs. AS + olanzapine $^{*}p < 0.05$ in post-hoc analyses. At post-pubertal stage PD 65-70, locomotor activity similarly significantly different amongst treatment groups: $F(3,54)= 4.521$ in the one-way analysis of variance:sense vs. AS $^{*}p < 0.05$, and AS vs. AS + olanzapine $^{*}p < 0.05$ in post-hoc analyses (**Figure 20A**).

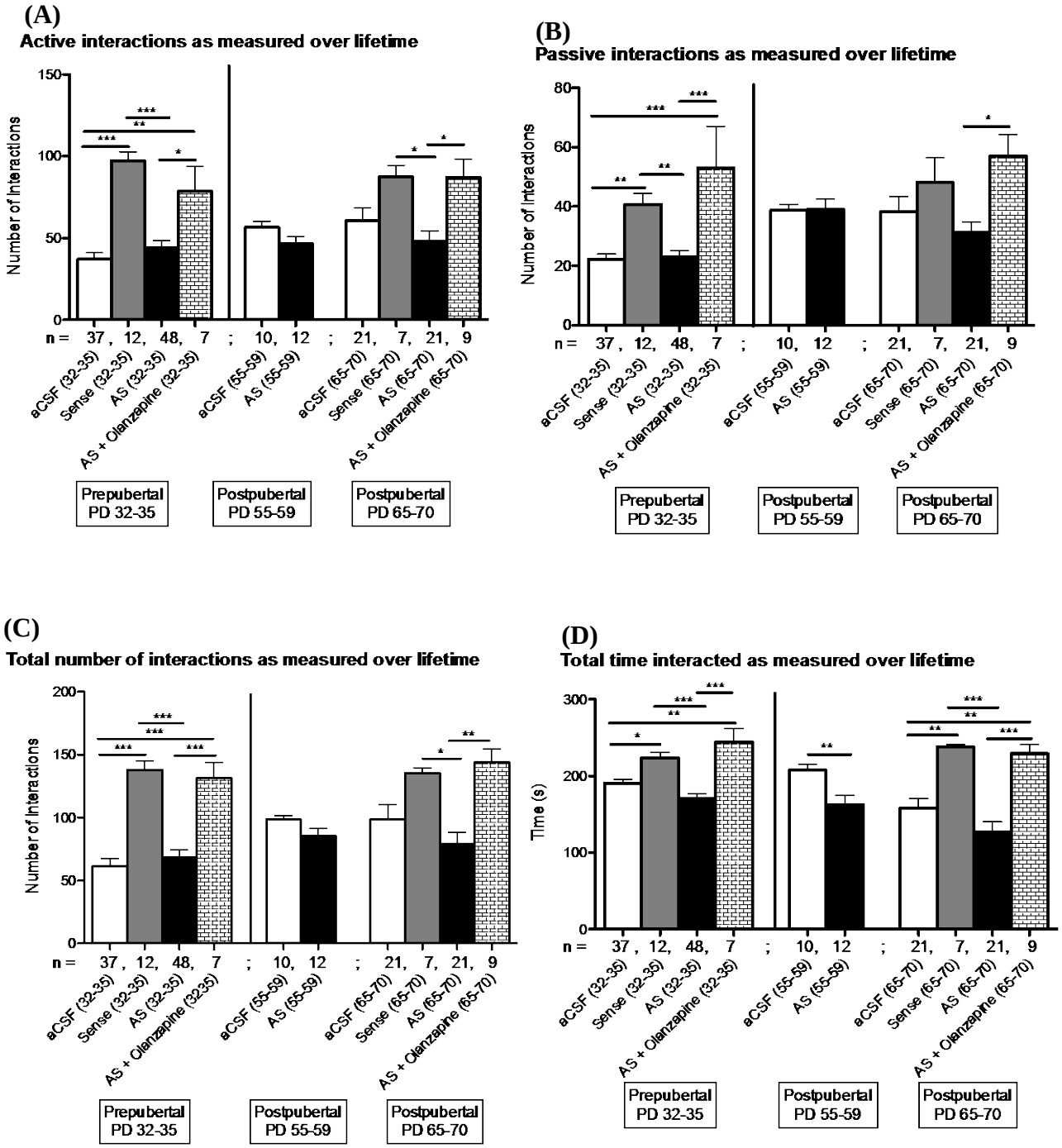
When measuring the number of passive interactions, synapsin II knockdown AS-treated rats displayed a decrease in social interaction as when compared to sense-treated and AS + olanzapine-treated animals at pre-pubertal stage PD 32-35, but a decrease in

activity was only detected in the synapsin II knockdown rat when compared to AS + olanzapine treatment at post-pubertal stage PD 65-70. A decrease in social interaction was found in aCSF-treated animals when compared to sense-treated and AS + olanzapine-treated animals at pre-pubertal stage PD 32-35. At pre-pubertal stage PD 32-35, social interaction was significantly different amongst treatment groups: $F(3,99)$: $=12.44$ in the one-way analysis of variance; aCSF vs. sense $^{**}p < 0.01$, aCSF vs. AS + olanzapine $^{***}p < 0.001$, sense vs. AS $^{**}p < 0.01$, and AS vs. AS + olanzapine $^{***}p < 0.001$ in post-hoc analyses. At post-pubertal stage PD 65-70, social interaction was similarly significantly different amongst treatment groups: $F(3,54) = 3.752$ in the one-way analysis of variance: AS vs. AS + olanzapine $^{*}p < 0.05$ (**Figure 20B**).

When measuring the total number of interactions, synapsin II knockdown AS-treated rats displayed a decrease in social interaction as when compared to sense-treated and AS + olanzapine-treated animals at pre-pubertal stage PD 32-35, and a decrease in activity was similarly detected in the synapsin II knockdown rat when compared to sense-treatment and AS + olanzapine treatment at post-pubertal stage PD 65-70. A decrease in social interaction was found in aCSF-treated animals when compared to sense-treated and AS + olanzapine-treated animals at pre-pubertal stage PD 32-35. At pre-pubertal stage PD 32-35, social interaction was significantly different amongst treatment groups: $F(3,100)$: $=18.02$ in the one-way analysis of variance; aCSF vs. sense $^{***}p < 0.001$, aCSF vs. AS + olanzapine $^{***}p < 0.001$, sense vs. AS $^{***}p < 0.001$, and AS vs. AS + Olanzapine $^{***}p < 0.001$ in post-hoc analyses. At post-pubertal stage PD 65-70, locomotor activity similarly significantly different amongst treatment groups: $F(3,54) =$

6.093 in the one-way analysis of variance: sense vs. AS *p < 0.05, and AS vs. AS + olanzapine **p < 0.01 (**Figure 20C**).

When measuring the total time interacted, synapsin II knockdown AS-treated rats displayed a decrease in social interaction as when compared to sense-treated and AS + olanzapine-treated animals at pre-pubertal stage PD 32-35 and at post-pubertal stage PD 65-70. Synapsin II knockdown AS-treated rats also display a significant decrease in social interaction when compared to aCSF-treated controls at post-pubertal stage PD 55-59. A decrease in social interaction was found in aCSF-treated animals when compared to sense-treated and AS + olanzapine-treated animals at pre-pubertal stage PD 32-35 and at post-pubertal stage PD 65-70. At pre-pubertal stage PD 32-35, social interaction was significantly different amongst treatment groups: F(3,100): =12.86 in the one-way analysis of variance; aCSF vs. sense *p < 0.05, aCSF vs. AS + olanzapine **p < 0.01, sense vs. AS ***p < 0.001, and AS vs. AS + olanzapine ***p < 0.001 in post-hoc analyses. At post-pubertal stage PD 55-59, social interaction aCSF vs. AS was significantly different as measured by a student's t-test: t(20) = 3.048, p = 0.0063. At post-pubertal stage PD 65-70, locomotor activity similarly significantly different amongst treatment groups: F(3,54) = 12.67 in the one-way analysis of variance: aCSF vs. sense **p < 0.01, aCSF vs AS + olanzapine **p < 0.01, sense vs. AS ***p < 0.001, and AS vs. AS + Olanzapine **p< 0.01 in post-hoc analyses (**Figure 20D**).


(A)
Active interactions as measured over lifetime
Number of Interactions
n = 37, 12, 48, 7 ; 10, 12 ; 21, 7, 21, 9
aCSF (32-35)
Sense (32-35)
AS (32-35)
AS + Olanzapine (32-35)
aCSF (55-59)
AS (55-59)
aCSF (65-70)
Sense (65-70)
AS (65-70)
AS + Olanzapine (65-70)
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70
(B)
Passive interactions as measured over lifetime
Number of Interactions
(C)
Total number of interactions as measured over lifetime
Number of Interactions
(D)
Total time interacted as measured over lifetime
Time (s)

Figures 20A-D: Following developmental synapsin II knockdown (PD 17-23), social interactions was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages. Graph indicates social interaction as measured (**A-C**) by number of interactions and (**D**) total time interacted in seconds (s) over a 5 minute recording period in aCSF-, sense-, AS-, and AS+olanzapine- treated animals. Synapsin II knockdown (AS-treated) animals were found to be significantly less social when compared to aCSF-treated and AS + olanzapine-treated animals. aCSF-treated animals were significantly less social than sense-treated and AS + olanzapine-treated animals. The significant decrease in social interaction was measured at pre-pubertal stages and persisted into post-pubertal stages. Treatment with antipsychotic drug, olanzapine, was found to significantly attenuate the decrease in social interaction in synapsin II knockdown animals. Sample size within each treatment group is indicated below the x-axis of the graph. When measuring active interactions (**A**), $F(3,99) = 16.18$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ at PD 32-35. At 65-70, $F(3,54) = 4.521$, $^{*}p < 0.05$. When measuring passive interactions (**B**), $F(3,99) = 12.44$, $^{**}p < 0.01$, $^{***}p < 0.001$ at PD 32-35. At 65-70, $F(3,54) = 3.752$, $^{*}p < 0.05$.When measuring total number of interactions (**C**), $F(3,100) = 18.02$, $^{***}p < 0.001$ at PD 32-35. At 65-70, $F(3,54) = 6.093$, $^{*}p < 0.05$, $^{**}p < 0.01$.When measuring total time interacted (**D**), $F(3,100) = 12.86$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ at PD 32-35. At PD 55-59, $t(20) = 3.048$, $p = 0.0063$, $^{**}p < 0.01$. At 65-70, $F(3,54) = 12.67$, $^{**}p < 0.01$, $^{***}p < 0.001$. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

4.3.2.1. Effect of acute antipsychotic drug olanzapine treatment on social interaction

At pre-pubertal stage PD 32-35, AS treatment caused a 55% decrease (***p < 0.001) in number of active interactions when compared to sense treatment, and a 19% increase when compared to aCSF treatment. This AS-induced decrease in social interaction was attenuated by a 78% increase (*p < 0.5) in active interactions with olanzapine treatment. AS-treatment caused a 43% decrease (**p < 0.01) in number of passive interactions when compared to sense treatment, and a 4% increase when compared to aCSF treatment. This AS-induced decrease in social interaction was attenuated by a 130% increase (***p < 0.001) in passive interactions with olanzapine treatment. AS-treatment caused a 51% decrease (***p < 0.001) in total number of interactions when compared to sense treatment, and a 11% increase when compared to aCSF treatment. This AS-induced decrease in total number of interactions was attenuated by a 93% increase (***p < 0.001) in total number of interactions with olanzapine treatment. AS-treatment caused a 24% decrease (***p < 0.001) in total time interacted when compared to sense treatment, and a 10% decrease when compared to aCSF treatment. This AS-induced decrease in total number of interactions was attenuated by a 43% increase (***p < 0.001) in total number of interactions with olanzapine treatment (**Figures 20A-D**).

At post-pubertal stage PD 65-70, AS treatment caused a 45% decrease (*p < 0.05) in number of active interactions when compared to sense treatment, and a 21% decrease when compared to aCSF treatment in number of passive interactions. This AS-induced decrease in social interaction was attenuated by a 82% increase (*p < 0.05) in active

interactions with olanzapine treatment. AS treatment caused a 35% decrease in number of passive interactions when compared to sense-treatment, and a 18% decrease when compared to aCSF treatment. This AS-induced decrease in social interaction was attenuated by a 83% increase (*$p < 0.05$) in passive interactions with olanzapine treatment. AS-treatment caused a 42% decrease (*$p < 0.05$) in total number of interactions when compared to sense treatment, and a 20% decrease when compared to aCSF treatment. This AS-induced decrease in total number of interactions was attenuated by a 82% increase (**$p < 0.01$) in total number of interactions with olanzapine treatment. AS-treatment caused a 47% decrease (***$p < 0.001$) in total time interacted when compared to sense treatment, and a 20% decrease when compared to aCSF treatment. This AS-induced decrease in total number of interactions was attenuated by a 81% increase (***$p < 0.001$) in total number of interactions with olanzapine treatment (**Figures 20A-D**).

4.3.2.2 Effect of gender on social interaction

In order to identify the effects of the gender differences of social interaction resulting from neurodevelopmental synapsin II knockdown, social interaction was analyzed in the male and female rats separately. Various parameters of social interaction were reported at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70) stages (**Figures 21A-D, 22A-D, 23A-D**). A significant difference was found in both the number of interactions and time spent interacting in both male and female populations.

Both populations show differing degrees of robustness at different stages of development and types of interactions.

In the male population, a significant decrease in active interactions was found in AS-treated rats as when compared to sense-treated animals at pre-pubertal stage PD 32-35. A decrease in number of active interactions was also found in aCSF-treated animals when compared to sense-treated and AS + olanzapine-treated animals at pre-pubertal stage PD 32-35. At pre-pubertal stage PD 32-35, number of active interactions was significantly different amongst treatment groups: $F(3,54)$: = 14.13 in the one-way analysis of variance; aCSF vs. sense ***$p < 0.001$, aCSF vs. AS + Olanzapine *$p < 0.05$, and sense vs. AS ***$p < 0.001$ in post-hoc analyses (**Figure 21A**). With respect to passive interactions, a significant decrease in number of passive interactions was found in AS-treated rats as when compared to AS + olanzapine-treated rats at PD 32-35. A decrease in number of passive interactions was also found in aCSF-treated animals when compared to both sense-treated and AS + olanzapine-treated animals at pre-pubertal stage PD 32-35. At pre-pubertal stage PD 32-35, number of active interactions was significantly different amongst treatment groups: $F(3,56)$: = 8.116 in the one-way analysis of variance; aCSF vs. sense *$p < 0.05$, aCSF vs. AS + olanzapine ***$p < 0.001$, and AS vs. AS + olanzapine **$p < 0.01$ in post-hoc analyses (**Figure 21B**). With respect to the total number of interactions, a significant decrease in number of interactions was found in AS-treated rats as when compared to sense-treated and AS + olanzapine-treated rats at PD 32-35. A decrease in number of interactions was also found in aCSF-treated animals when compared to both sense-treated and AS + olanzapine-treated animals at

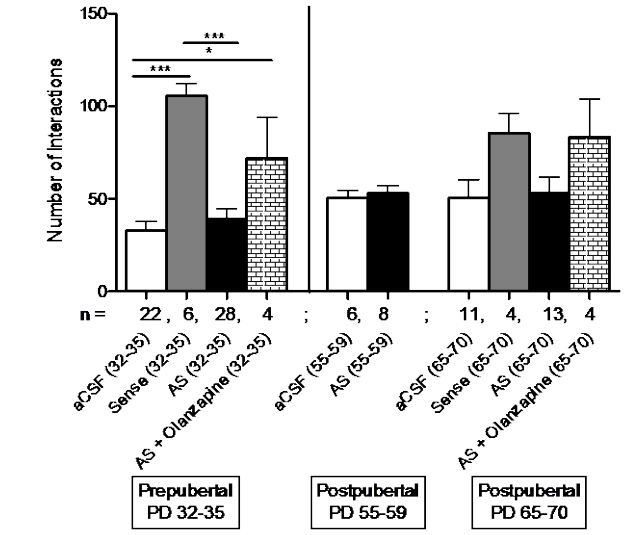
pre-pubertal stage PD 32-35. At pre-pubertal stage PD 32-35, number of total interactions was significantly different amongst treatment groups: $F(3,56): = 11.03$ in the one-way analysis of variance; aCSF vs. sense $^{***}p < 0.001$, aCSF vs. AS + Olanzapine $^{*}p < 0.05$, sense vs. AS $^{***}p < 0.001$, and AS vs. AS + olanzapine $^{*}p < 0.05$ in post-hoc analyses (**Figure 21C**). With respect to the total time spent interacting, a significant decrease in amount of time was found in AS-treated rats as when compared to sense-treated and AS + olanzapine-treated rats at PD 32-35. This decrease in time spent interacting was again found in AS-treated animals when compared to aCSF-treated animals at PD 55-59, and as when compared to sense-treated animals at PD 65-70. At pre-pubertal stage PD 32-35, time spent interacting was significantly different amongst treatment groups: $F(3,56): = 9.752$ in the one-way analysis of variance; sense vs. AS $^{***}p < 0.001$, and AS vs. AS + olanzapine $^{**}p < 0.01$ in post-hoc analyses. At PD 55-59, $t(12) = 3.314$, $p = 0.0062$, $^{**}p < 0.01$. At PD 65-70, time spent interacting was significantly different amongst treatment groups: $F(3,28): = 4.464$ in the one-way analysis of variance; sense vs. AS $^{*}p < 0.05$ in post-hoc analyses (**Figure 21D**).

In the female population, a significant decrease in active interactions was found in AS-treated rats as when compared to sense-treated animals at pre-pubertal stage PD 32-35. A decrease in number of active interactions was also found in aCSF-treated animals when compared to sense-treated and AS + olanzapine-treated animals at pre-pubertal stage PD 32-35. A significant decrease in active interactions was also found in female AS-treated rats as when compared to aCSF-treated rats at PD 55-59. At pre-pubertal stage PD 32-35, number of active interactions was significantly different amongst treatment

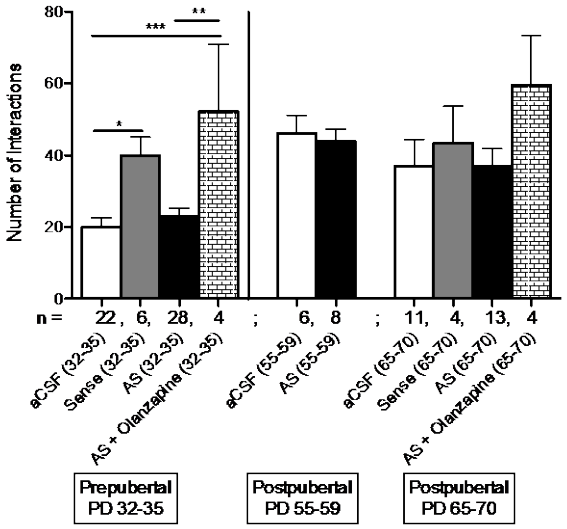
groups: F(3,39): = 6.564 in the one-way analysis of variance; aCSF vs. sense **p < 0.01, aCSF vs. AS + olanzapine *p < 0.05, and sense vs. AS **p < 0.01 in post-hoc analyses. At PD 55-59, t(6) = 4.048, p = 0.0067, **p < 0.01 (**Figure 22A**). With respect to passive interactions, a significant decrease in number of passive interactions was only found in AS-treated rats as when compared to AS + olanzapine-treated rats at PD 65-70. At pre-pubertal stage PD 65-70, number of passiveinteractions was significantly different amongst treatment groups: F(3,21): = 3.714 in the one-way analysis of variance; AS vs. AS + olanzapine *p < 0.05 in post-hoc analyses (**Figure 22B**). With respect to the total number of interactions, a significant decrease in number of interactions was found in AS-treated rats as when compared to sense-treated and AS + olanzapine-treated rats at PD 32-35. A decrease in number of total interactions was also found in aCSF-treated animals when compared to both sense-treated and AS + olanzapine-treated animals at pre-pubertal stage PD 32-35. At PD 55-59, a significant decrease in total number of interactions was found in AS-treated female rats as compared to aCSF-treated female rats. At PD 65-70, a significant decrease in number of interactions was found in AS-treated rats as when compared to sense-treated and AS + olanzapine-treated rats. At pre-pubertal stage PD 32-35, total number of interactions was significantly different amongst treatment groups: F(3,40): = 6.744 in the one-way analysis of variance; aCSF vs. sense **p < 0.01, aCSF vs. AS + olanzapine *p < 0.05, sense vs. AS *p < 0.05, and AS vs. AS + olanzapine *p < 0.05 in post-hoc analyses.At post-pubertal stage PD 55-59, total number of interactions of aCSF vs. AS was significantly different: t(6) = 8.172, p = 0.0002, ***p < 0.0001. At post-pubertal stage PD 65-70, total number of interactions was significantly different

amongst treatment groups: $F(3,22)$: = 5.767 in the one-way analysis of variance; sense vs. AS *$p < 0.05$, and AS vs. AS + olanzapine **$p < 0.01$ in post-hoc analyses (**Figure 22C**). With respect to the total time spent interacting, a significant decrease in amount of time was found in AS-treated rats as when compared to AS + olanzapine-treated rats at PD 32-35. This decrease in time spent interacting was again found in AS-treated animals when compared to aCSF-treated animals at PD 55-59. A significant decrease in time spent interaction was found in AS-treated animals as when compared to aCSF-treated, AS + olanzapine-treated, and sense-treated animals at PD 65-70. At pre-pubertal stage PD 32-35, time spent interacting was significantly different amongst treatment groups: $F(3,40)$: = 4.209 in the one-way analysis of variance; AS vs. AS + olanzapine *$p < 0.05$ in post-hoc analyses. At PD 55-59, $t(6) = 6.930$, $p = 0.0004$, ***$p < 0.001$. At PD 65-70, time spent interacting was significantly different amongst treatment groups: $F(3,21)$: = 11.74 in the one-way analysis of variance; aCSF vs. sense *$p < 0.05$, aCSF vs. AS + olanzapine *$p < 0.05$, sense vs. AS **$p < 0.01$, and AS vs. AS + olanzapine ***$p < 0.001$ in post-hoc analyses (**Figure 22D**).

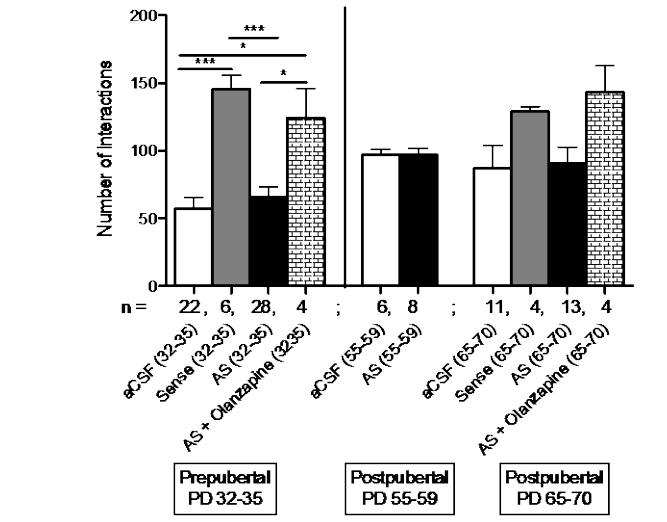
(A)


Active interactions in male rats as measured over lifetime
Number of Interactions
150
100
50
0
***
***
*
n = 22, 6, 28, 4 ; 6, 8 ; 11, 4, 13, 4
aCSF (32-35)
Sense (32-35)
AS (32-35)
AS + Olanzapine (32-35)
aCSF (55-59)
AS (55-59)
aCSF (65-70)
Sense (65-70)
AS (65-70)
AS + Olanzapine (65-70)
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70

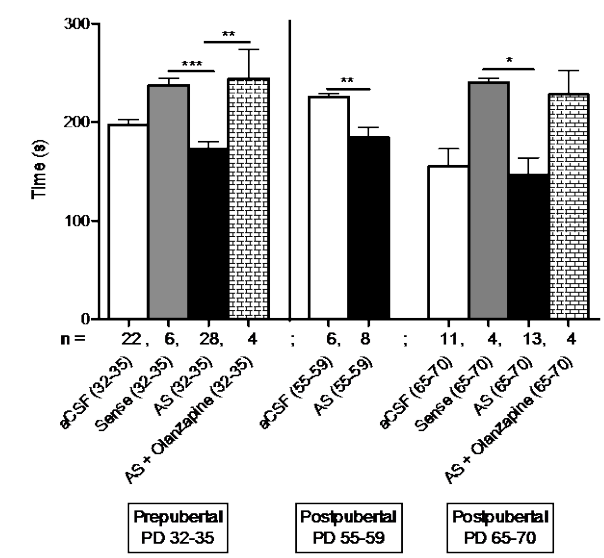
(B)


Passive interactions in male rats as measured over lifetime
Number of Interactions
80
60
40
20
0
***
**
*
n = 22, 6, 28, 4 ; 6, 8 ; 11, 4, 13, 4
aCSF (32-35)
Sense (32-35)
AS (32-35)
AS + Olanzapine (32-35)
aCSF (55-59)
AS (55-59)
aCSF (65-70)
Sense (65-70)
AS (65-70)
AS + Olanzapine (65-70)
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70

(C)

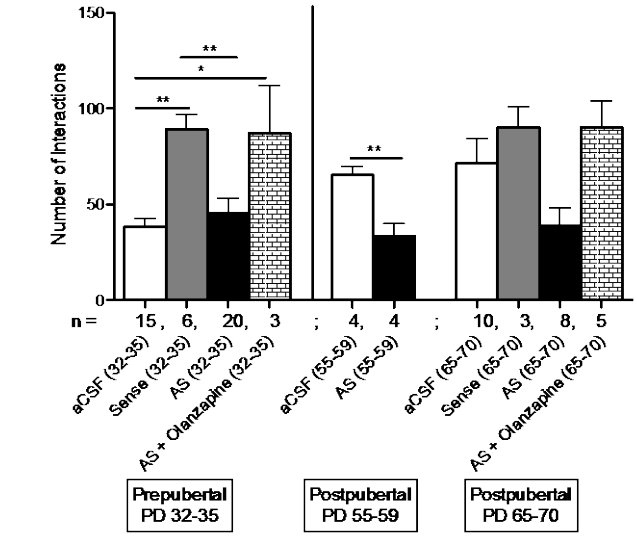

Total number of interactions in male rats as measured over lifetime
Number of Interactions
200
150
100
50
0
***
*
***
*
n = 22, 6, 28, 4 ; 6, 8 ; 11, 4, 13, 4
aCSF (32-35)
Sense (32-35)
AS (32-35)
AS + Olanzapine (3235)
aCSF (55-59)
AS (55-59)
aCSF (65-70)
Sense (65-70)
AS (65-70)
AS + Olanzapine (65-70)
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70

(D)

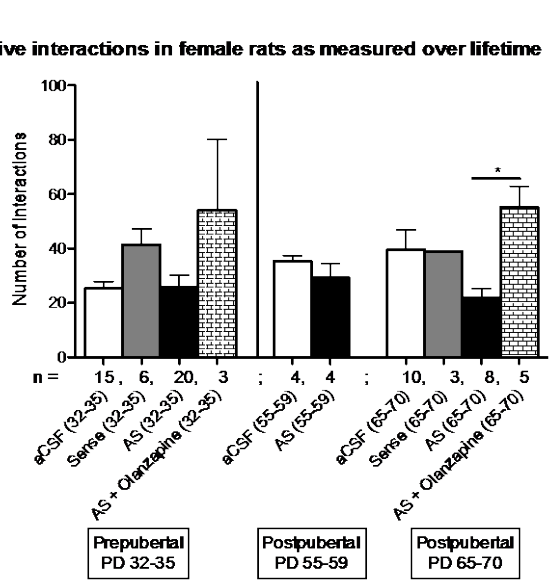

Total time interacted in male rats as measured over lifetime
Time (s)
300
200
100
0
***
**
**
*
n = 22, 6, 28, 4 ; 6, 8 ; 11, 4, 13, 4
aCSF (32-35)
Sense (32-35)
AS (32-35)
AS + Olanzapine (32-35)
aCSF (55-59)
AS (55-59)
aCSF (65-70)
Sense (65-70)
AS (65-70)
AS + Olanzapine (65-70)
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70

Figures 21A-D: Following developmental synapsin II knockdown (PD 17-23), locomotor activity was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70) stages in male animals. Graphs indicates (**A-C**) number of interactions and (**D**) time spent interacting as measured in seconds (s) in aCSF-, sense-, AS-, and AS + olanzapine- treated female animals. Synapsin II knockdown (AS-treated) animals were found to be significantly less social when compared to sense-treated control animals at select stages. A significant decrease in social interaction was measured at pre-pubertal stages and persisted into post-pubertal stages. Treatment with antipsychotic drug, olanzapine, was found to attenuate thisdecrease in social interaction. Sample size within each treatment group is indicated below the x-axis of the graph. (**A**) For active interactions, $F(3,54): = 14.13$ at PD 32-35. (**B**) For passive interactions, $F(3,56): = 8.116$ at PD 32-35. (**C**) For total number of interactions, $F(3,56): = 11.03$ at PD 32-35. (**D**) For total time spent interacting, $F(3,56): = 9.752$ at PD 32-35, $t(12) = 3.314$, $p = 0.0062$ at PD 55-59, and $F(3,28): = 4.464$ at PD 65-70. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. $*p<0.05$; $**p<0.01$, $***p < 0.001$.

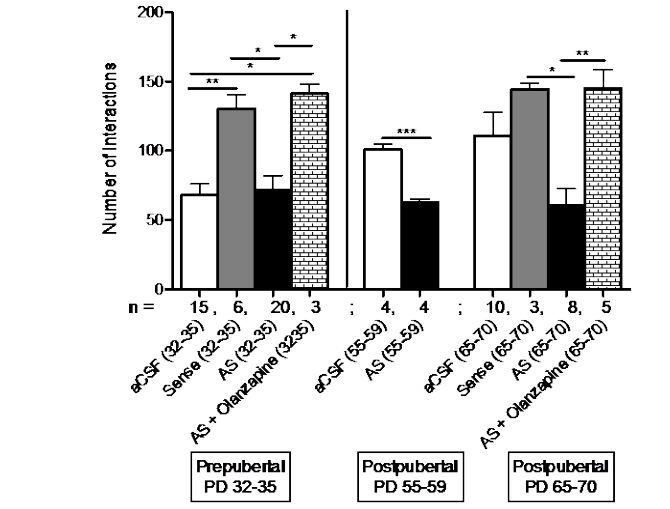
(A)


Active interactions in female rats as measured over lifetime
Number of Interactions
150
100
50
0
n = 15, 6, 20, 3 ; 4, 4 ; 10, 3, 8, 5
aCSF (32-35)
Sense (32-35)
AS (32-35)
AS + Olanzapine (32-35)
aCSF (55-59)
AS (55-59)
aCSF (65-70)
Sense (65-70)
AS (65-70)
AS + Olanzapine (65-70)
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70

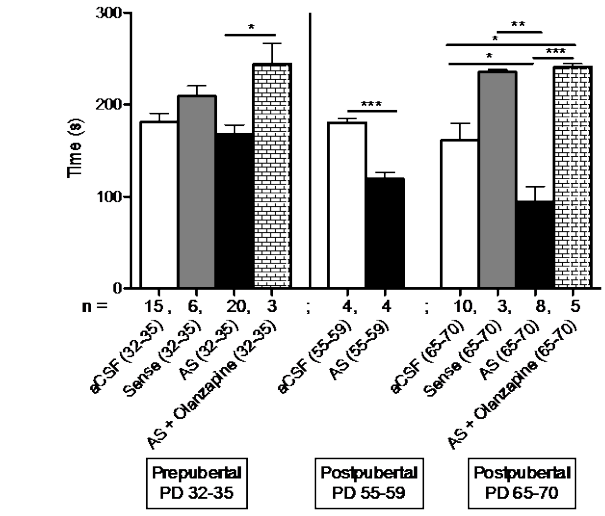
(B)


Passive interactions in female rats as measured over lifetime
Number of Interactions
100
80
60
40
20
0
n = 15, 6, 20, 3 ; 4, 4 ; 10, 3, 8, 5
aCSF (32-35)
Sense (32-35)
AS (32-35)
AS + Olanzapine (32-35)
aCSF (55-59)
AS (55-59)
aCSF (65-70)
Sense (65-70)
AS (65-70)
AS + Olanzapine (65-70)
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70

(C)


Total number of interactions in female rats as measured over lifetime
Number of Interactions
200
150
100
50
0
n = 15, 6, 20, 3 ; 4, 4 ; 10, 3, 8, 5
aCSF (32-35)
Sense (32-35)
AS (32-35)
AS + Olanzapine (3235)
aCSF (55-59)
AS (55-59)
aCSF (65-70)
Sense (65-70)
AS (65-70)
AS + Olanzapine (65-70)
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70

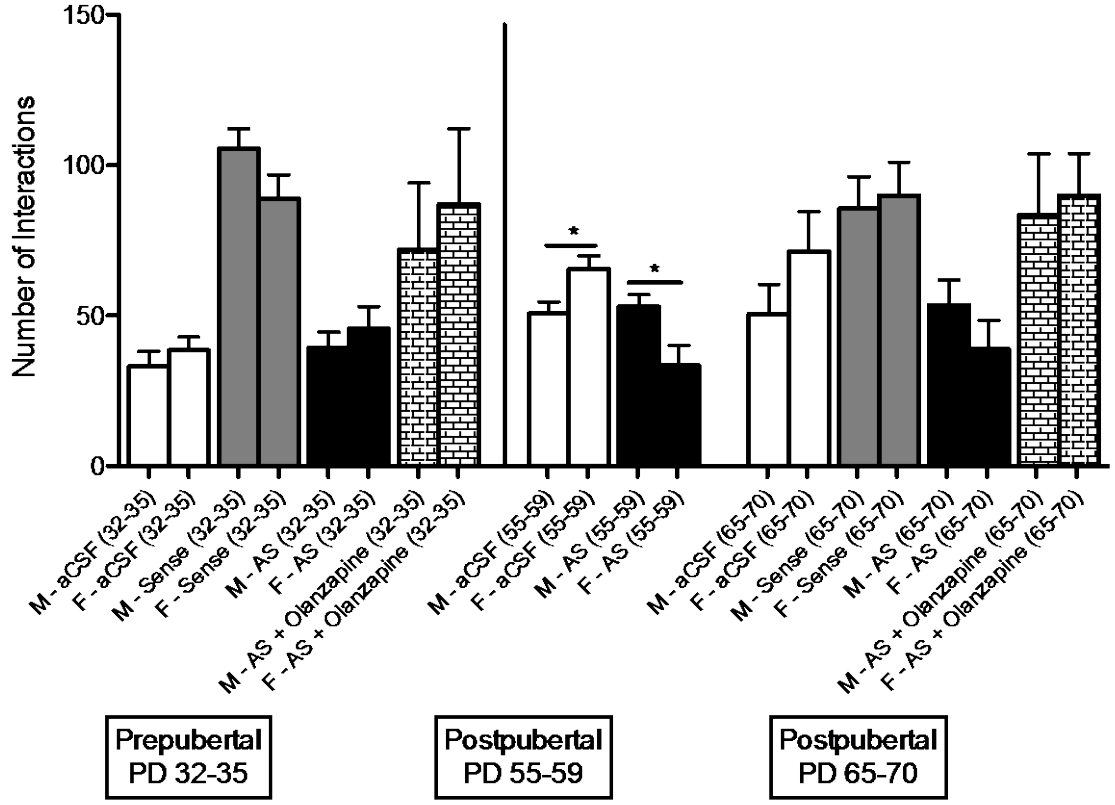
(D)


Total time interacted in female rats as measured over lifetime
Time (s)
300
200
100
0
n = 15, 6, 20, 3 ; 4, 4 ; 10, 3, 8, 5
aCSF (32-35)
Sense (32-35)
AS (32-35)
AS + Olanzapine (32-35)
aCSF (55-59)
AS (55-59)
aCSF (65-70)
Sense (65-70)
AS (65-70)
AS + Olanzapine (65-70)
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70

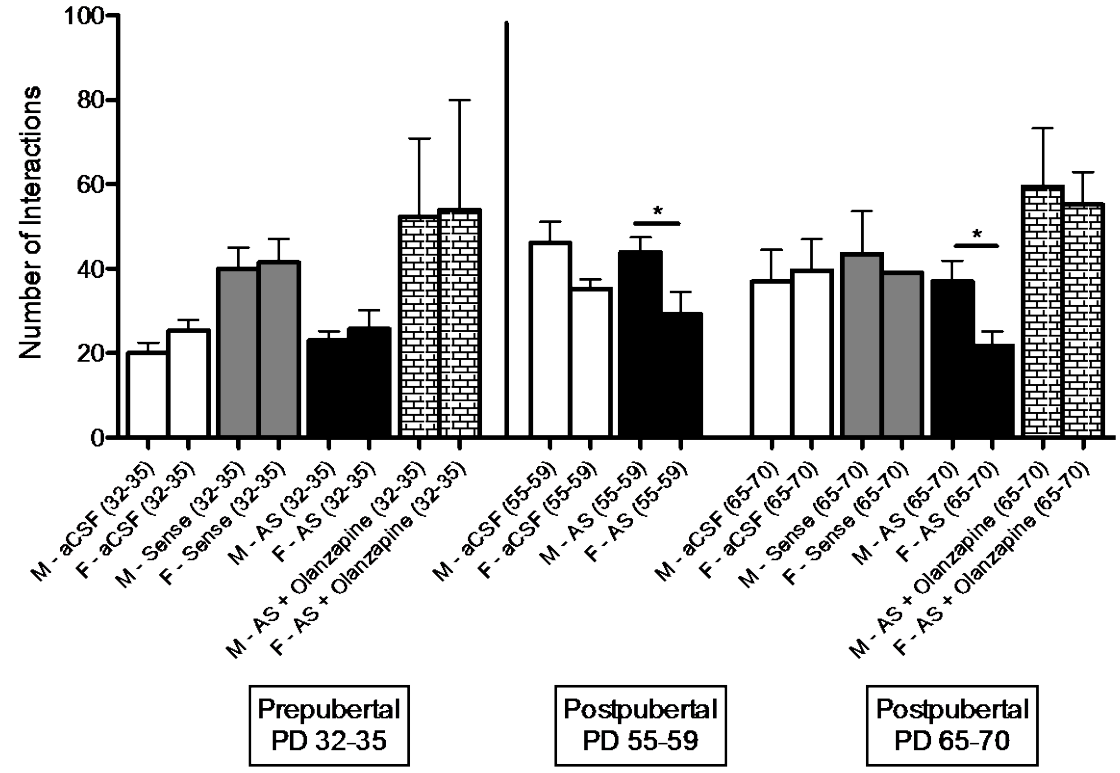
Figures 22A-D: Following developmental synapsin II knockdown (PD 17-23), locomotor activity was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70) stages in female animals. Graphs indicates (**A**-C) number of interactions and (**D**) time spent interacting as measured in seconds (s) in aCSF-, sense-, AS-, and AS + olanzapine- treated female animals. Synapsin II knockdown (AS-treated) animals were found to be significantly less social when compared to aCSF-treated and sense-treated control animals at select stages. A significant decrease in social interaction was measured at pre-pubertal stages and persisted into post-pubertal stages. Treatment with antipsychotic drug, olanzapine, was found to attenuate this decrease in social interaction. Sample size within each treatment group is indicated below the x-axis of the graph. (**A**) For active interactions, $F(3,39): = 6.564$ at PD 32-35 and $t(6) = 4.048$, $p = 0.0067$ at PD 55-59. (**B**) For passive interactions, $F(3,21): = 3.714$ at PD 65-70. (**C**) For total number of interactions, $F(3,40): = 6.744$ at PD 32-35, $t(6) = 8.172$, $p = 0.0002$ at PD 55-59, and $F(3,22): = 5.767$ at PD 65-70(**D**) For total time spent interacting, $F(3,40): = 4.209$ at PD 32-35, $t(6) = 6.930$, $p = 0.0004$ at PD 55-59, and $F(3,21): = 11.74$ at PD 65-70. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. $*p<0.05$; $**p<0.01$, $***p < 0.001$.

A few gender differences were observed amongst the various treatment groups. At post-pubertal stage PD 55-59, active interactions of AS-treated male vs. female was significantly different as measured by a student's t-test: $t(10) = 2.638$, $p = 0.0248$. Active interactions of aCSF-treated male vs. female at PD 55-59 was also significantly different as measured by student's t-test: $t(8) = 2.432$, $p = 0.0411$ (**Figure 23A**). Passive interactions at PD 55-59 and PD 65-70 was found to be significantly different in AS-treated male vs. female animals, as measured by student's t-test: $t(10) = 2.365$, $p = 0.0396$ and $t(19) = 2.259$, $p = 0.0359$, respectively (**Figure 23B**). Total number of interactions of AS-treated male vs. female was significantly different at PD 55-59, as measured by student's t-test:$t(10) = 4.772$, $p = 0.0008$. Total number of interactions of sense-treated male vs. female was significantly different at PD 65-70, as measured by student's t-test:$t(5) = 2.739$, $p = 0.0409$ (**Figure 23C**). Total time spent interacting was significantly different in aCSF-treated and AS-treated and male vs. female animals at PD 55-59, as measured by student's t-test:$t(8) = 8.729$, $p = < 0.0001$ and $t(10) = 4.113$, $p = 0.0021$, respectively (**Figure 22D**).

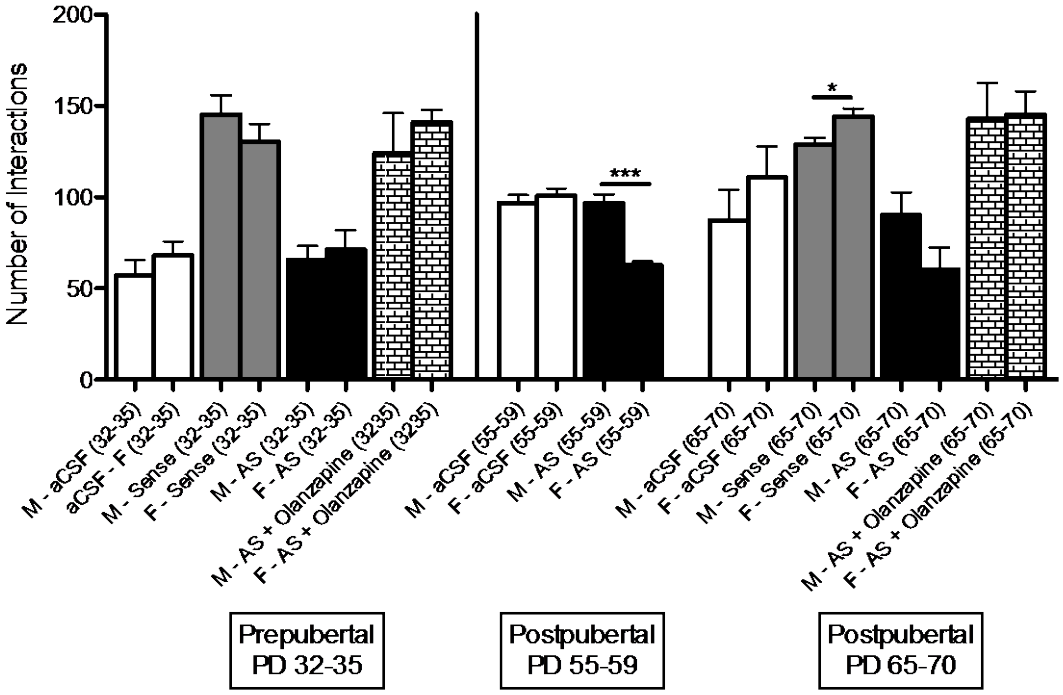
(A) Comparison of active interactions between males and females as measured over lifetime



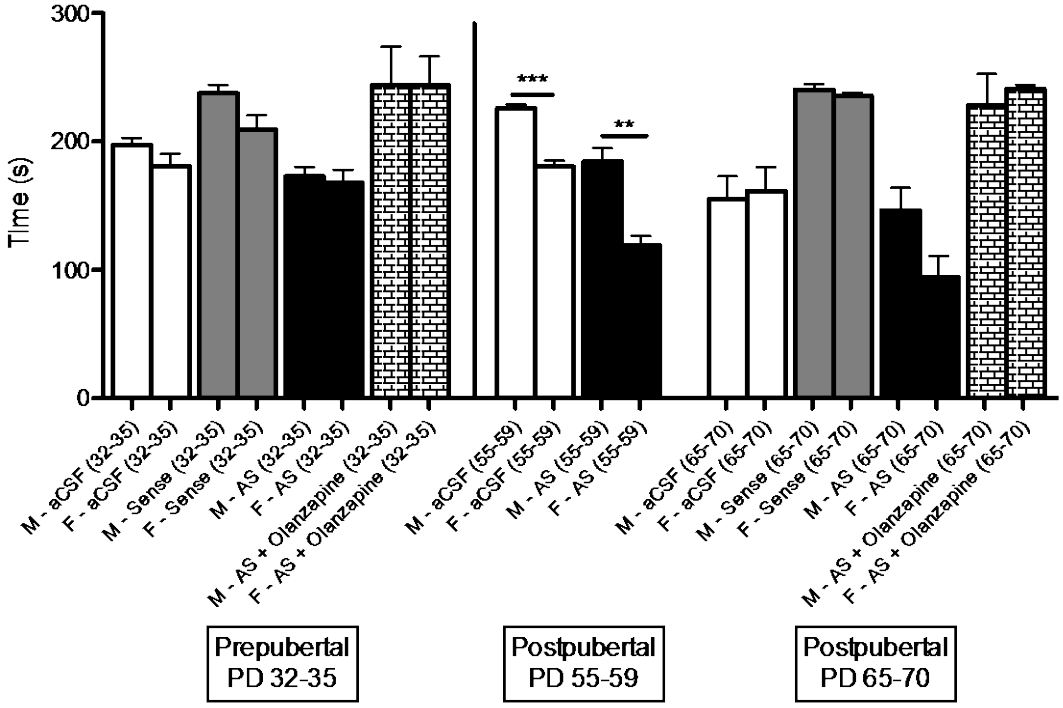
(B) Comparison of passive interactions between males and females as measured over lifetime



(C) Comparison of total number of interactions between males and females as measured over lifetime



(D) Comparison of total time interacted between males and females as measured over lifetime

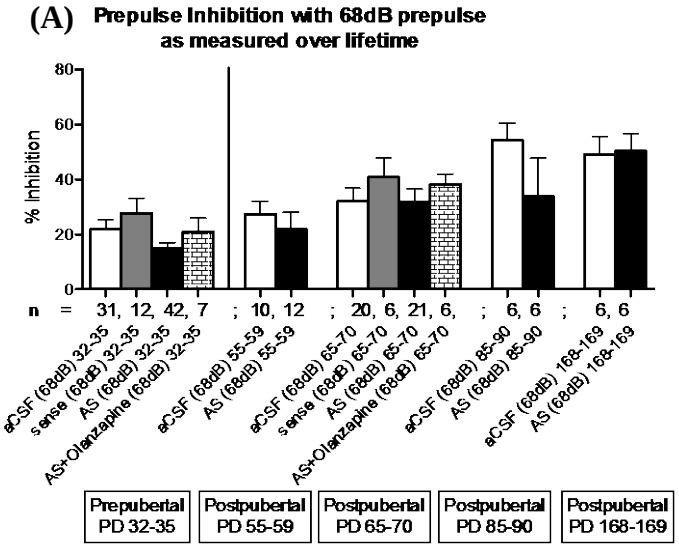


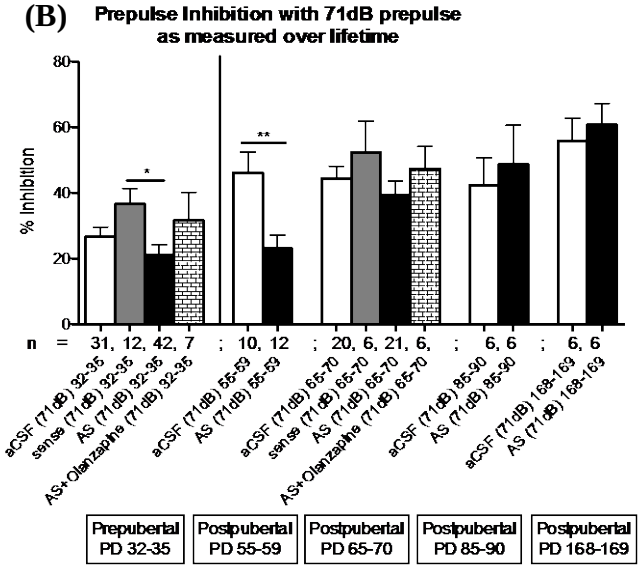
Figures 23A-D: An analysis of gender differences between the various treatment groups was considered, as a function of the developmental timeline. Social interaction was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70) stages, and compared between male (M) and female (F) animals. Graph indicates (**A-C**) total number of interactions and (**D**) total time spent interacting as measured in seconds (s) over a 5 minute recording period in aCSF-, sense-, AS-, and AS + olanzapine- treated female animals. (**A**) Active interactions at PD 55-59: $t(8) = 2.432$, $p = 0.0411$in aCSF-treated male vs. female, $t(10) = 2.638$, $p = 0.0248$ in AS-treated male vs. female. (**B**) Passive interactions in AS-treated male vs. female: $t(10) = 2.365$, $p = 0.0396$ ad PD 55-59 and $t(19) = 2.259$, $p = 0.0359$ at PD 65-70. (**C**) Total number of interactions:$t(10) = 4.772$, $p = 0.0008$ in AS-treated male vs. female at PD 55-59 and t-test: $t(5) = 2.739$, $p = 0.0409$ in sense-treated male vs. female at PD 65-70. (**D**) Total time spent interaction at PD 55-59: $t(8) = 8.729$, $p = < 0.0001$ in aCSF-treated male vs. female and $t(10) = 4.113$, $p = 0.0021$ in AS-treated male vs. female. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar.

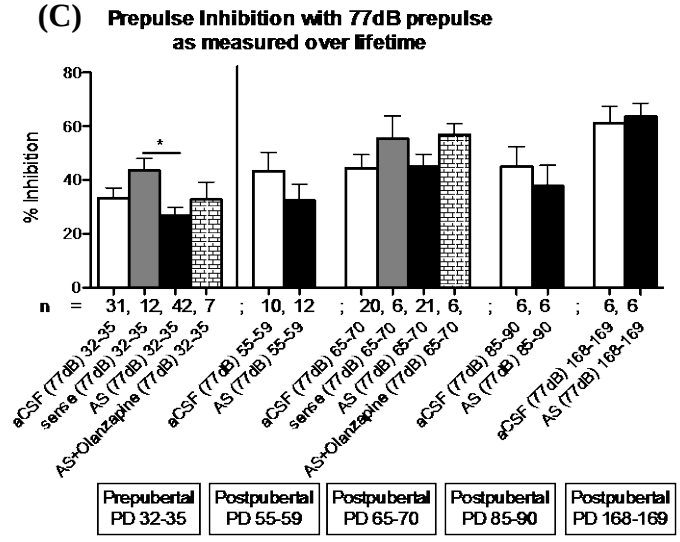
4.3.3. Effect of PD 17-23 synapsin II knockdown on prepulse inhibition

Following 7 consecutive days of neurodevelopmental synapsin II knockdown (PD 17-23), prepulse inhibition was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages (**Figures 24A-C**). Alterations in prepulse inhibition with various prepulse decibels (i.e. 68dB, 71dB, 77dB) were observed resulting from the various treatments (**Figure 24A-C**). The alterations in prepulse inhibition were, however, not robust at both pre-pubertal and post-pubertal stages through all prepulse decibels.

With the presentation of a 68dB prepulse prior a 120dB pulse, there were no noticeable changes in prepulse inhibition amongst the different treatment groups, and across all stages of development (**Figure 24A**). With the presentation of a 71dB prepulse prior a 120dB pulse, a deficit in prepulse inhibition was observed as a result of synapsin II knockdown (AS treatment) as compared to sense- and aCSF- treated animals at pre-pubertal stage PD 32-35 and post-pubertal stage PD 55-59, respectively. At PD 32-35, $F(3,80): = 2.854$ in the one-way analysis of variance; sense vs. AS $*p < 0.05$ in post-hoc analyses. At PD 55-59, prepulse inhibition of aCSF vs. AS was significantly different as measured by a student's t-test: $t(17) = 3.229$, $p = 0.0049$, $**p < 0.01$ (**Figure 24B**). With the presentation of a 77dB prepulse prior a 120dB pulse, a deficit in prepulse inhibition was observed as in AS-treated animals as compared to sense-treated control animals at PD 32-35, and deficits did not persist into post-pubertal stages. At PD 32-35, $F(3,78): = 2.571$ in the one-way analysis of variance; sense vs. AS $*p < 0.05$ in post-hoc analyses (**Figure 24C**).


(A) Prepulse Inhibition with 68dB prepulse as measured over lifetime
% Inhibition
n = 31, 12, 42, 7 ; 10, 12 ; 20, 6, 21, 6, ; 6, 6 ; 6, 6
aCSF (68dB) 32-35
sense (68dB) 32-35
AS (68dB) 32-35
AS+Olanzapine (68dB) 32-35
aCSF (68dB) 55-59
AS (68dB) 55-59
aCSF (68dB) 65-70
sense (68dB) 65-70
AS (68dB) 65-70
AS+Olanzapine (68dB) 65-70
aCSF (68dB) 85-90
AS (68dB) 85-90
aCSF (68dB) 168-169
AS (68dB) 168-169
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70
Postpubertal PD 85-90
Postpubertal PD 168-169


(B) Prepulse Inhibition with 71dB prepulse as measured over lifetime
% Inhibition
n = 31, 12, 42, 7 ; 10, 12 ; 20, 6, 21, 6, ; 6, 6 ; 6, 6
aCSF (71dB) 32-35
sense (71dB) 32-35
AS (71dB) 32-35
AS+Olanzapine (71dB) 32-35
aCSF (71dB) 55-59
AS (71dB) 55-59
aCSF (71dB) 65-70
sense (71dB) 65-70
AS (71dB) 65-70
AS+Olanzapine (71dB) 65-70
aCSF (71dB) 85-90
AS (71dB) 85-90
aCSF (71dB) 168-169
AS (71dB) 168-169
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70
Postpubertal PD 85-90
Postpubertal PD 168-169


(C) Prepulse Inhibition with 77dB prepulse as measured over lifetime
% Inhibition
n = 31, 12, 42, 7 ; 10, 12 ; 20, 6, 21, 6, ; 6, 6 ; 6, 6
aCSF (77dB) 32-35
sense (77dB) 32-35
AS (77dB) 32-35
AS+Olanzapine (77dB) 32-35
aCSF (77dB) 55-59
AS (77dB) 55-59
aCSF (77dB) 65-70
sense (77dB) 65-70
AS (77dB) 65-70
AS+Olanzapine (77dB) 65-70
aCSF (77dB) 85-90
AS (77dB) 85-90
aCSF (77dB) 168-169
AS (77dB) 168-169
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70
Postpubertal PD 85-90
Postpubertal PD 168-169

Figures 24A-C: Following developmental synapsin II knockdown (PD 17-23), prepulse inhibition was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages. Graph indicates percent of prepulse inhibition with the presentation of prepulses (**A**) 68dB, (**B**) 71dB, and (**C**) 77dB prior a 120dB pulse in aCSF-, sense-, AS-, and AS + olanzapine- treated animals. Synapsin II knockdown (AS-treated) animals were found to manifest deficits in prepulse inhibition when compared to aCSF-treated and sense-treated animals. At select prepulse decibels, deficits were measured at pre-pubertal stages and persisted into post-pubertal stages at select prepulse decibels. Treatment with antipsychotic drug, olanzapine, was found to prevent prepulse deficits induced by synapsin II knockdown. Sample size within each treatment group is indicated below the x-axis of the graph. (**A**) No significant changes were detected at prepulse decibel 68dB. (**B**) At prepulse decibel 71dB, $F(3,80): = 2.854$ at PD 32-35 and $t(17) = 3.229$, $p = 0.0049$ at PD 55-59. (**C**) At prepulse decibel 77dB, $F(3,78): = 2.571$ at PD 32-35. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. *$p<0.05$; **$p < 0.01$; ***$p<0.001$.

4.3.3.1. Effect of acute antipsychotic drug olanzapine treatment on prepulse inhibition

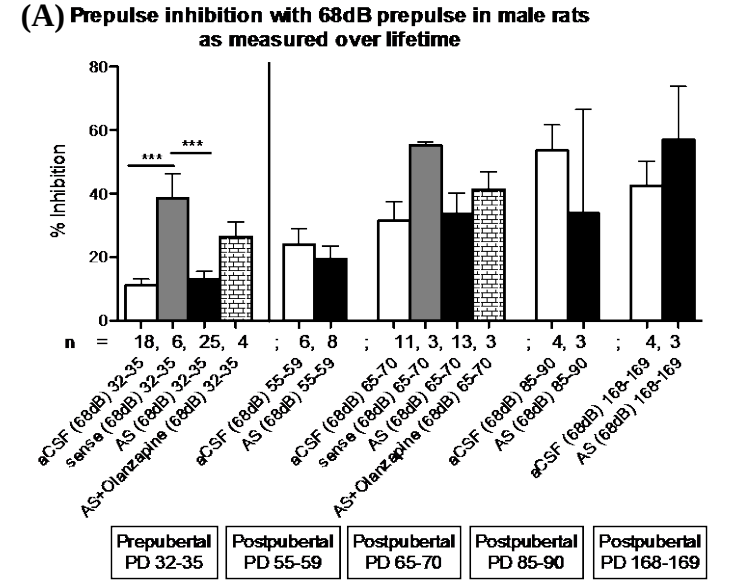
At a prepulse decibel of 68dB, olanzapine treatment had no significant effect on AS-treatment (**Figure 24A**). At a prepulse decibel of 71dB at pre-pubertal PD 32-35 stage, AS treatment induced a 41% decrease (*$p < 0.5$) in prepulse inhibition as compared to sense treatment, and a 27% decrease as compared to aCSF treatment. Olanzapine treatment attenuated this deficit with a 49% increase in prepulse inhibition as compared to AS-treated group (**Figure 24B**). At a prepulse decibel of 77dB at pre-pubertal PD 32-35 stage, AS treatment induced a 39% decrease (*$p < 0.5$) in prepulse inhibition as compared to sense treatment, and a 19% decrease as compared to aCSF treatment. Olanzapine treatment attenuated this deficit with a 22% increase in prepulse inhibition as compared to AS-treated group (**Figure 24C**).

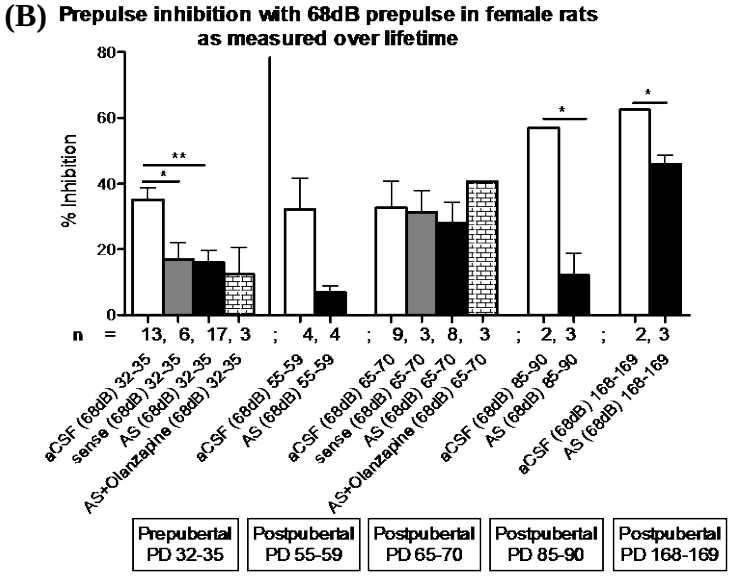
4.3.3.2 Effect of gender on prepulse inhibition

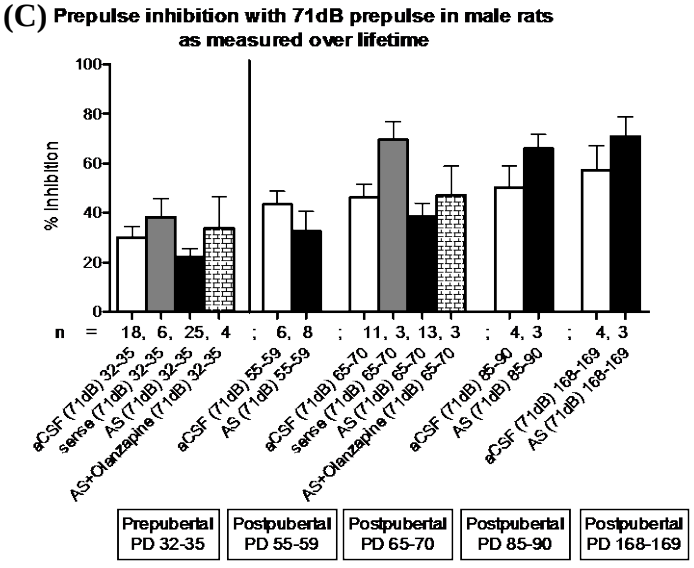
In order to identify the effects of the gender differences of prepulse inhibition resulting from neurodevelopmental synapsin II knockdown, prepulse inhibition was also analyzed in the male and female rat populations separately. Responses to various prepulse decibels (i.e. 68dB, 71dB, 77dB) were reported at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages (**Figures 25A-F**). A significant difference was found in prepulse inhibition across varying stages of development in both male and female populations. Both populations show differing degrees of robustness at different stages of development and types of interactions.

At 68dB, a significant deficit in prepulse inhibition was detected in AS-treated rats as when compared to sense-treated rats at pre-pubertal stage PD 32-35 of the male population. aCSF treated animals also show a deficit in prepulse inhibition as when compared to sense treatment at PD 32-35 of the male population. 68dB at PD 32-35 of male population: $F(3,36): = 11.04$ in the one-way analysis of variance; aCSF vs. sense $***p < 0.001$, and sense vs. AS $***p < 0.001$ in post-hoc analyses (**Figure 25A**). Within the female population, prepulse inhibition deficits were found in AS-treated animals as compared to aCSF treatment at pre-pubertal stage PD 32-35, and post-pubertal stages PD 85-90 and PD 168-169. Prepulse inhibition deficits were also found in sense treatment as compared to aCSF treatment at pre-pubertal stage PD 32-35. 68dB at PD 32-35 of female population: $F(3,26): = 5.201$ in the one-way analysis of variance; aCSF vs. sense $*p < 0.05$, and aCSF vs. AS $**p < 0.01$ in post-hoc analyses. 68dB at PD 85-90 and PD 168-169 of female population, prepulse inhibition of aCSF vs. AS was significantly different

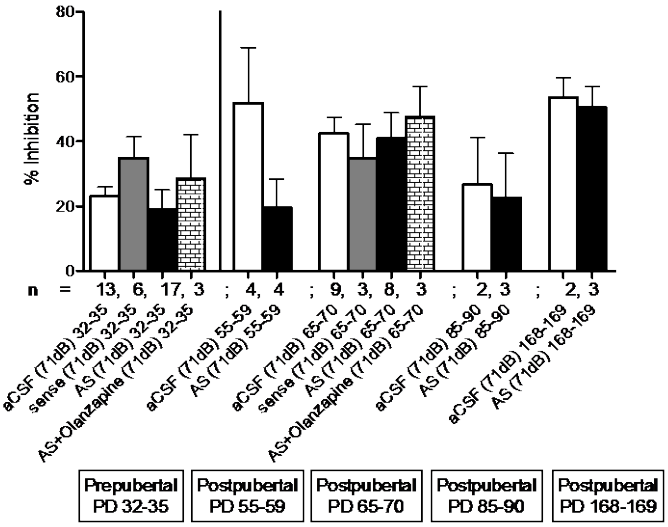
as measured by a student's t-test: $t(3) = 5.290$, $p = 0.0132$, $*p < 0.05$ and $t(3) = 4.787$, $p = 0.0173$, $*p < 0.05$, respectively (**Figure 25B**). No significant differences in prepulse inhibition was found with the presentation of a 71dB prepulse at both pre-pubertal and post-pubertal stages, in both male and female populations (**Figure 25C-D**). At 77dB, no significant deficits in prepulse inhibition were detected at both pre-pubertal and post-pubertal stages of the male population (**Figure 25E**). A significant deficit in prepulse inhibition was found in AS-treated rats as compared to aCSF-treated rats at post-pubertal stage PD 168-169, as measured by a student's t-test: $t(3) = 5.290$, $p = 0.0132$, $*p < 0.05$ and $t(3) = 3.715$, $p = 0.0339$, $*p < 0.05$ (**Figure 25F**).


(A) Prepulse inhibition with 68dB prepulse in male rats as measured over lifetime
% Inhibition
n = 18, 6, 25, 4 ; 6, 8 ; 11, 3, 13, 3 ; 4, 3 ; 4, 3
aCSF (68dB) 32-35
sense (68dB) 32-35
AS (68dB) 32-35
AS+Olanzapine (68dB) 32-35
aCSF (68dB) 55-59
AS (68dB) 55-59
aCSF (68dB) 65-70
sense (68dB) 65-70
AS (68dB) 65-70
AS+Olanzapine (68dB) 65-70
aCSF (68dB) 85-90
AS (68dB) 85-90
aCSF (68dB) 168-169
AS (68dB) 168-169
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70
Postpubertal PD 85-90
Postpubertal PD 168-169

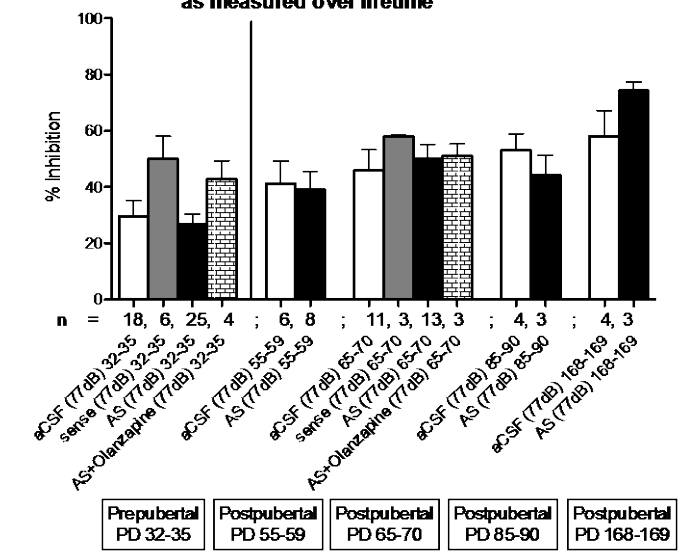

(B) Prepulse inhibition with 68dB prepulse in female rats as measured over lifetime
% Inhibition
n = 13, 6, 17, 3 ; 4, 4 ; 9, 3, 8, 3 ; 2, 3 ; 2, 3
aCSF (68dB) 32-35
sense (68dB) 32-35
AS (68dB) 32-35
AS+Olanzapine (68dB) 32-35
aCSF (68dB) 55-59
AS (68dB) 55-59
aCSF (68dB) 65-70
sense (68dB) 65-70
AS (68dB) 65-70
AS+Olanzapine (68dB) 65-70
aCSF (68dB) 85-90
AS (68dB) 85-90
aCSF (68dB) 168-169
AS (68dB) 168-169
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70
Postpubertal PD 85-90
Postpubertal PD 168-169


(C) Prepulse inhibition with 71dB prepulse in male rats as measured over lifetime
% Inhibition
n = 18, 6, 25, 4 ; 6, 8 ; 11, 3, 13, 3 ; 4, 3 ; 4, 3
aCSF (71dB) 32-35
sense (71dB) 32-35
AS (71dB) 32-35
AS+Olanzapine (71dB) 32-35
aCSF (71dB) 55-59
AS (71dB) 55-59
aCSF (71dB) 65-70
sense (71dB) 65-70
AS (71dB) 65-70
AS+Olanzapine (71dB) 65-70
aCSF (71dB) 85-90
AS (71dB) 85-90
aCSF (71dB) 168-169
AS (71dB) 168-169
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70
Postpubertal PD 85-90
Postpubertal PD 168-169

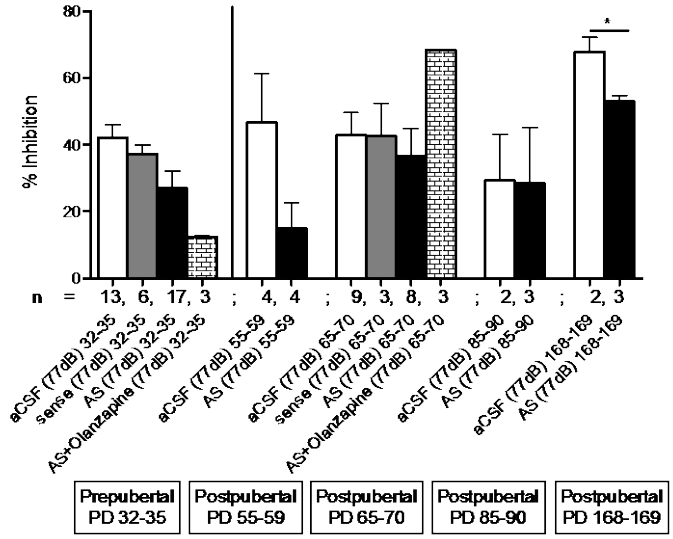
(D) Prepulse inhibition with 71dB prepulse in female rats as measured over lifetime



(E) Prepulse inhibition with 77dB prepulse in male rats as measured over lifetime

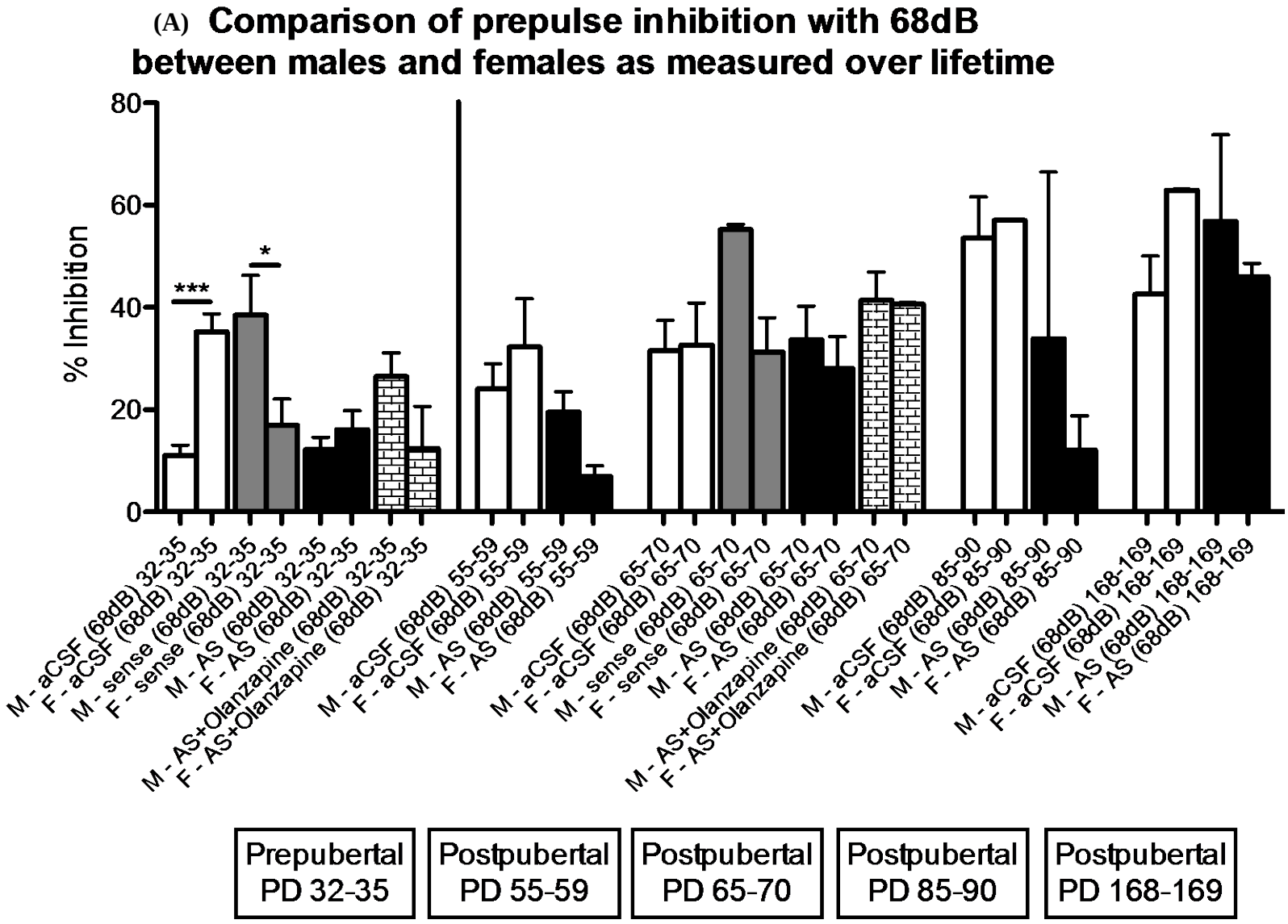


(F) Prepulse inhibition with 77dB prepulse in female rats as measured over lifetime

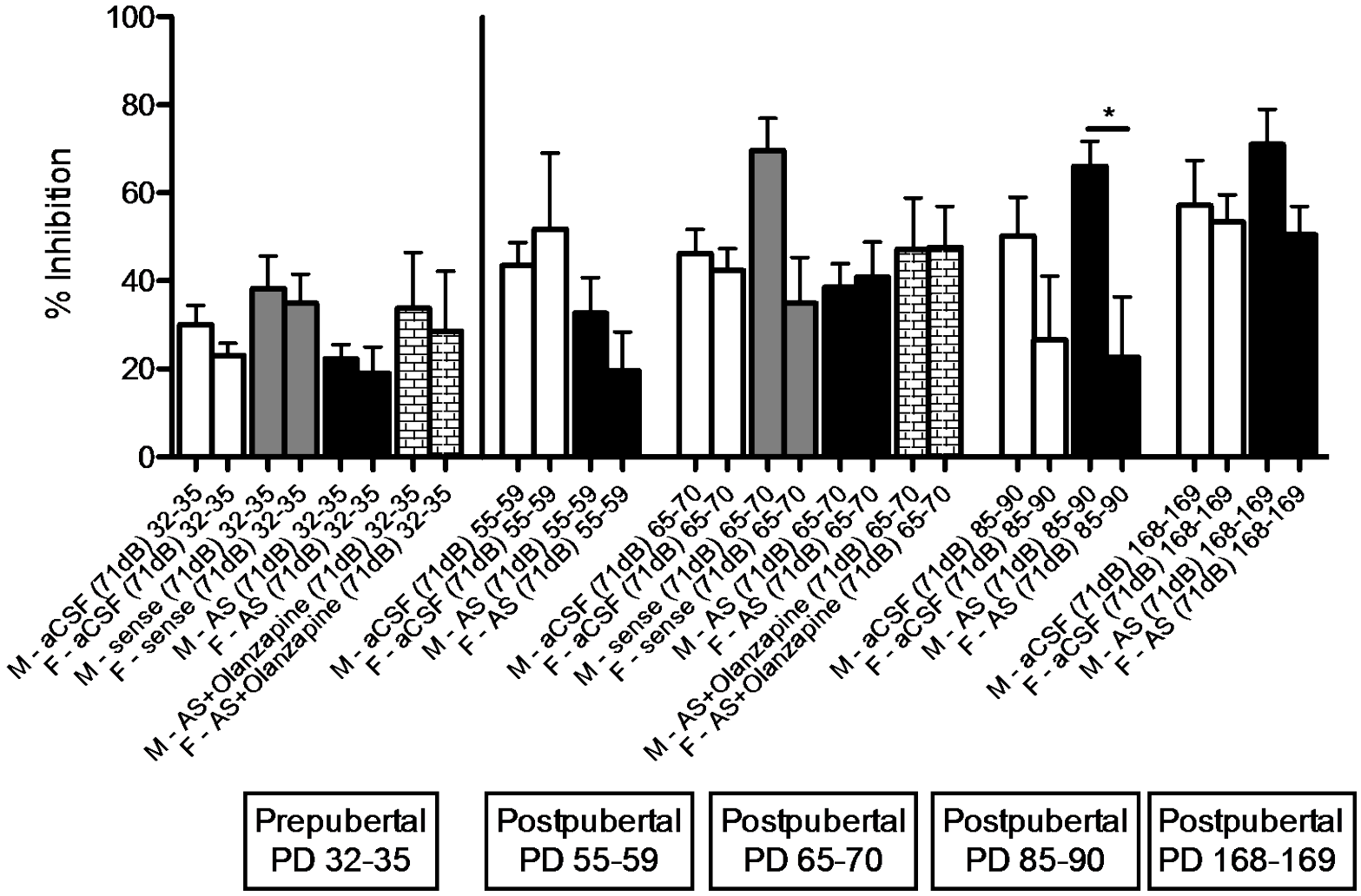


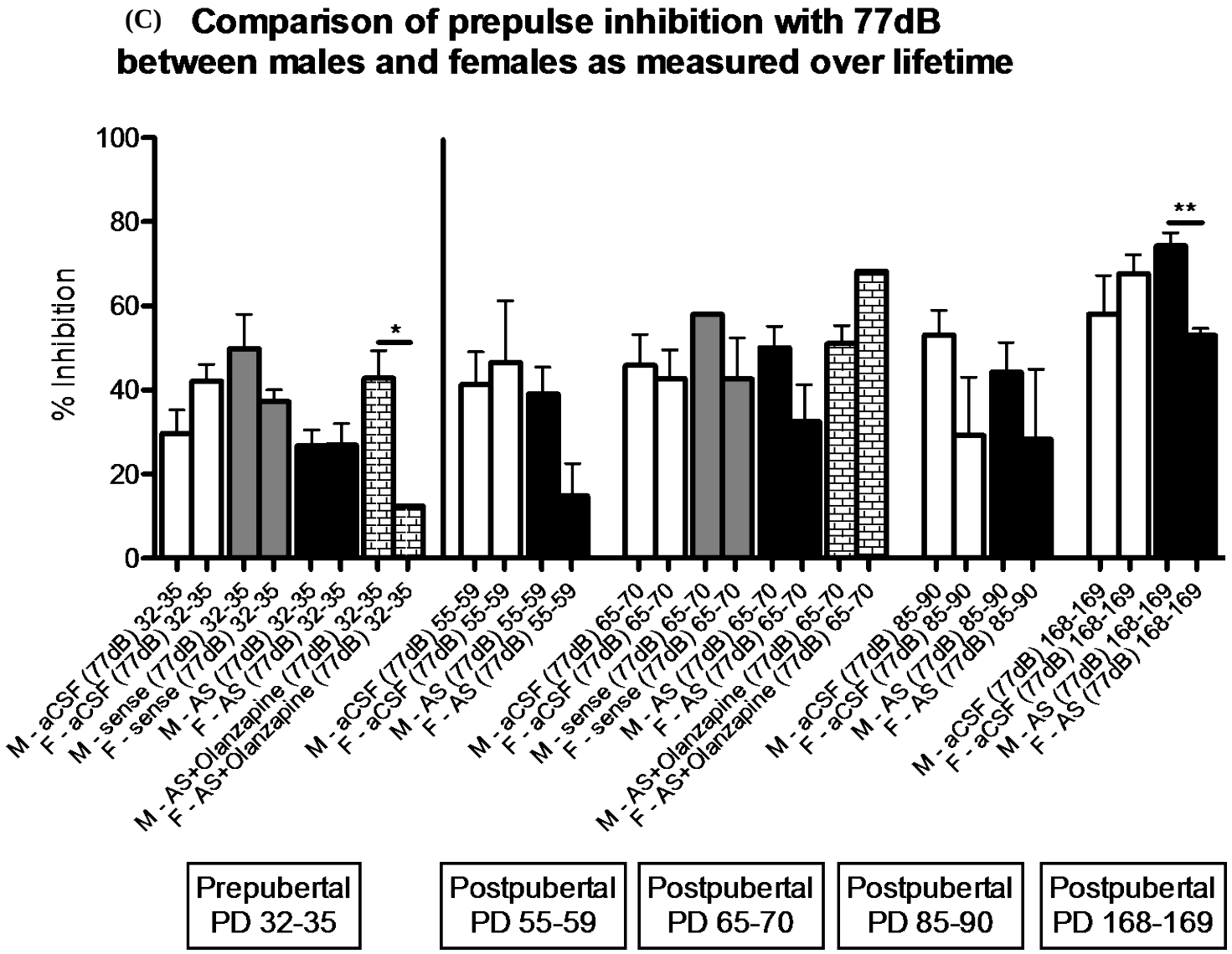
Figures 25A-F: Following developmental synapsin II knockdown (PD 17-23), locomotor activity was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages in male (**A, C, E**) and female (**B, D, F**) animals. Graphs indicates prepulse inhibition to 2 varying prepulses: 68dB (**A, B**), 71dB (**C, D**), and 77dB (**E, F**) in aCSF-, sense-, AS-, and AS + olanzapine- treated female animals. Deficits in prepulse inhibition resulting from synapsin II knockdown were found to vary according to developmental stages in both male and female populations. Treatment with antipsychotic drug, olanzapine, was found to attenuate deficits in prepulse inhibition. Sample size within each treatment group is indicated below the x-axis of the graph. (**A**) $F(3,36)$: = 11.04 at PD 32-35. (**B**) $F(3,26)$: = 5.201 at PD 32-35, $t(3) = 5.290$, $p = 0.0132$ at PD 85-90, and $t(3) = 4.787$, $p = 0.0173$ at PD 168-169. (**F**) $t(3) = 5.290$, $p = 0.0132$ at PD 168-169. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. *$p<0.05$; **$p<0.01$, ***$p < 0.001$.

A few gender differences were observed amongst the various treatment groups. Prepulse inhibition at 68dB of aCSF-treated and sense-treated male vs. female was significantly different at pre-pubertal stage PD 32-35,as measured by a student's t-test: $t(21) = 6.469$, $p < 0.0001$ and $t(10) = 2.321$, $p = 0.0427$, respectively (**Figure 26A**). Prepulse inhibition at 71dB of AS-treated male vs. female was significantly different at PD 85-90, as measured by a student's t-test: $t(3) = 3.458$, $p = 0.0407$ (**Figure 26B**). Prepulse inhibition at 77dB of AS + Olanzapine-treated male vs. female was significantly different at pre-pubertal stage PD 32-35 as measured by a student's t-test: $t(4) = 3.117$, $p = 0.0356$. Prepulse inhibition at 77dB of AS-treated male vs. female was significantly different at post-pubertal stage PD 168-169 as measured by a student's t-test: $t(4) = 6.020$, $p = 0.0038$ (**Figure 26C**).


(A) Comparison of prepulse inhibition with 68dB between males and females as measured over lifetime
% Inhibition
80
60
40
20
0
***
*
M - aCSF (68dB) 32-35
F - aCSF (68dB) 32-35
M - sense (68dB) 32-35
F - sense (68dB) 32-35
M - AS (68dB) 32-35
F - AS (68dB) 32-35
M - AS+Olanzapine (68dB) 32-35
F - AS+Olanzapine (68dB) 32-35
M - aCSF (68dB) 55-59
F - aCSF (68dB) 55-59
M - AS (68dB) 55-59
F - AS (68dB) 55-59
M - aCSF (68dB) 65-70
F - aCSF (68dB) 65-70
M - sense (68dB) 65-70
F - sense (68dB) 65-70
M - AS (68dB) 65-70
F - AS (68dB) 65-70
M - AS+Olanzapine (68dB) 65-70
F - AS+Olanzapine (68dB) 65-70
M - aCSF (68dB) 85-90
F - aCSF (68dB) 85-90
M - AS (68dB) 85-90
F - AS (68dB) 85-90
M - aCSF (68dB) 168-169
F - aCSF (68dB) 168-169
M - AS (68dB) 168-169
F - AS (68dB) 168-169
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70
Postpubertal PD 85-90
Postpubertal PD 168-169

(B) Comparison of prepulse inhibition with 71dB between males and females as measured over lifetime



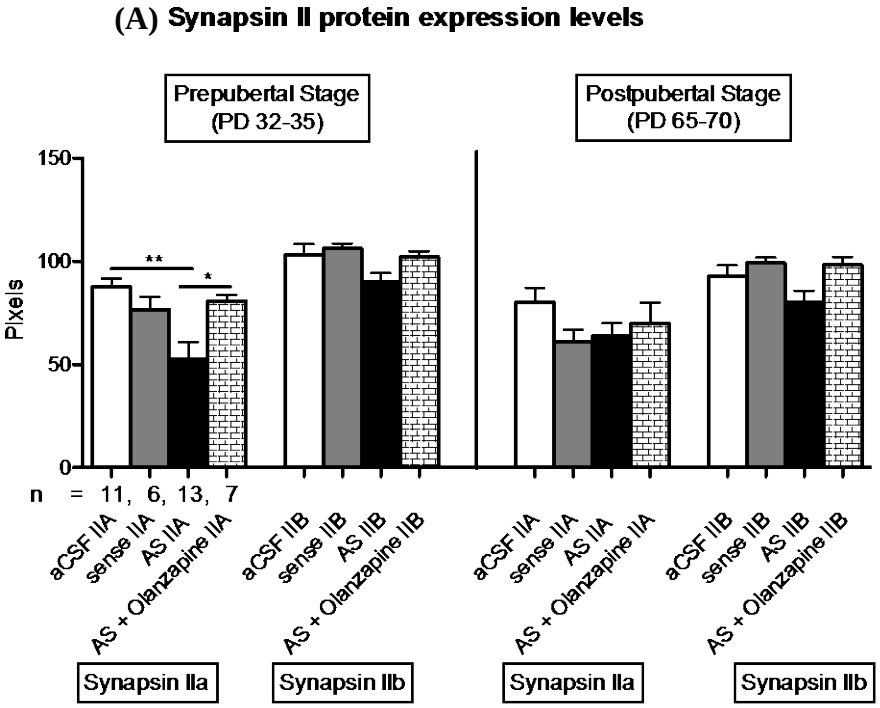

(C) Comparison of prepulse inhibition with 77dB between males and females as measured over lifetime
% Inhibition
100
80
60
40
20
0
*
**
M - aCSF (77dB) 32-35
F - aCSF (77dB) 32-35
M - sense (77dB) 32-35
F - sense (77dB) 32-35
M - AS (77dB) 32-35
F - AS (77dB) 32-35
M - AS+Olanzapine (77dB) 32-35
F - AS+Olanzapine (77dB) 32-35
M - aCSF (77dB) 55-59
F - aCSF (77dB) 55-59
M - AS (77dB) 55-59
F - AS (77dB) 55-59
M - aCSF (77dB) 65-70
F - aCSF (77dB) 65-70
M - sense (77dB) 65-70
F - sense (77dB) 65-70
M - AS (77dB) 65-70
F - AS (77dB) 65-70
M - AS+Olanzapine (77dB) 65-70
F - AS+Olanzapine (77dB) 65-70
M - aCSF (77dB) 85-90
F - aCSF (77dB) 85-90
M - AS (77dB) 85-90
F - AS (77dB) 85-90
M - aCSF (77dB) 168-169
F - aCSF (77dB) 168-169
M - AS (77dB) 168-169
F - AS (77dB) 168-169
Prepubertal PD 32-35
Postpubertal PD 55-59
Postpubertal PD 65-70
Postpubertal PD 85-90
Postpubertal PD 168-169

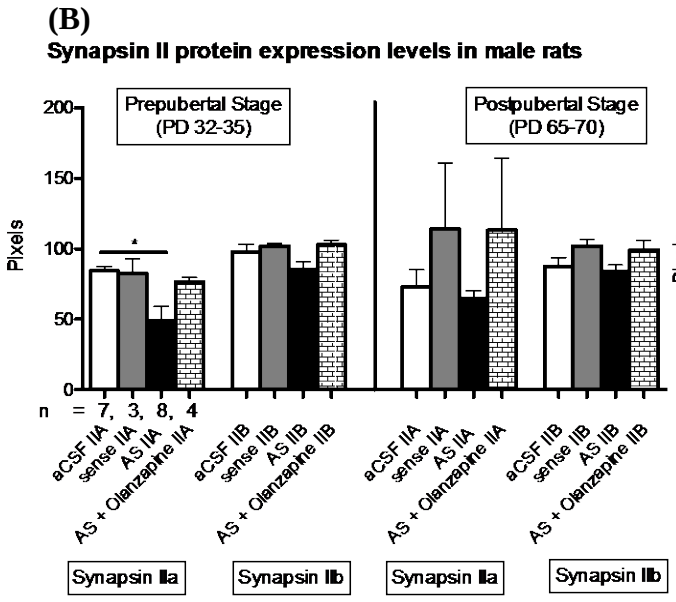
Figures 26A-C: An analysis of gender differences between the various treatment groups was considered, as a function of the developmental timeline. Prepulse inhibition was measured at both pre-pubertal (PD 32-35) and post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages, and compared between male (M) and female (F) animals. (**A**) 68dB at PD 32-35: $t(21) = 6.469$, $p < 0.0001$ in aCSF-treated male vs. female and $t(10) = 2.321$, $p = 0.0427$ in sense-treated male vs. female animals. (**B**) 71dB at PD 85-90: $t(3) = 3.458$, $p = 0.0407$ in AS-treated male vs. female animals. (**C**) 77dB at PD 32-35: $t(4) = 3.117$, $p = 0.0356$ in AS + olanzapine-treated male vs. female animals. 77dB at PD 32-35: $t(4) = 3.117$, $p = 0.0356$ in AS + olanzapine-treated male vs. female animals. 77dB at PD 168-169: $t(4) = 6.020$, $p = 0.0038$ in AS-treated male vs. female animals. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar.

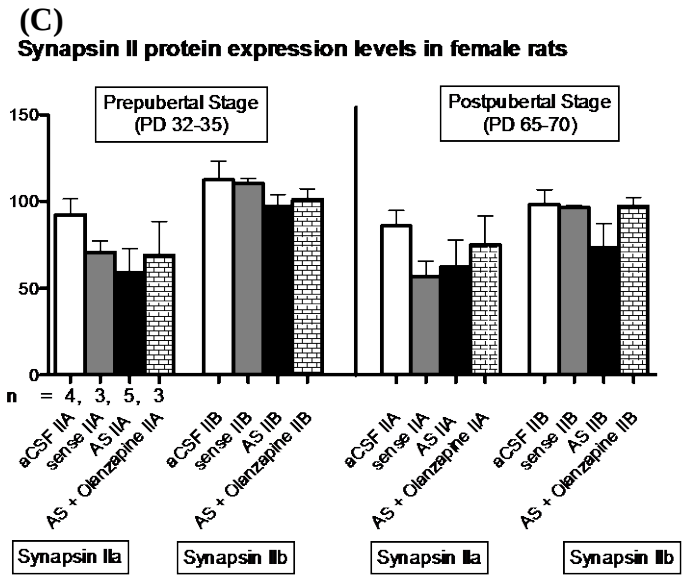
4.4. Effect of PD 17-23 synapsin II knockdown on medial prefrontal cortical brain circuitry and the effect of acute olanzapine treatment

4.4.1. Effect of PD 17-23 developmental knockdown on protein expression levels of synapsin II

Immunoblotting results revealed that synapsin II knockdown during PD 17-23 lasting reductions in synapsin isoform IIa into pre-pubertal stage PD 32-35, but these synapsin II a deficits did not persist into post-pubertal PD 65-70 stages. Olanzapine treatment was found to attenuate this decrease in synapsin IIa protein expression at pre-pubertal stage PD 32-35. Protein expression of synapsin IIb was not significantly altered in comparison to the other treatment groups at both pre-pubertal stage PD 32-35 and post-pubertal stage PD 65-70. Protein expression levels of synapsin IIa at PD 32-35: $F(2,26) = 8.547$ in one-way analysis of variance, aCSF vs. AS $^{**}p < 0.01$ and AS vs. AS + olanzapine $^{*}p < 0.05$ in post-hoc analyses (**Figure 27A**). At pre-pubertal stage PD 32-35, AS-treated animals showed a 40% decrease ($^{**}p < 0.01$) in synapsin IIa protein expression levels in comparison to aCSF-treated control animals. Olanzapine treatment was found to normalize this deficit in synapsin IIa protein expression levels with a 52% increase ($^{*}p < 0.05$) in comparison to AS-treated animals. Treated male animals displayed a similar significant decrease in synapsin IIa protein expression levels at pre-pubertal stage PD 32-35: $F(3,17) = 4.612$ in one-way analysis of variance, aCSF vs. AS $^{*}p < 0.05$ in post-hoc analysis (**Figure 27B**). Treated female animals display a similar trend but results were not significant (**Figure 27C**).


(A) Synapsin II protein expression levels
Prepubertal Stage (PD 32-35)
Postpubertal Stage (PD 65-70)
Pixels
150
100
50
0
**
*
n = 11, 6, 13, 7
aCSF IIA
sense IIA
AS IIA
AS + Olanzapine IIA
aCSF IIB
sense IIB
AS IIB
AS + Olanzapine IIB
Synapsin IIa
Synapsin IIb

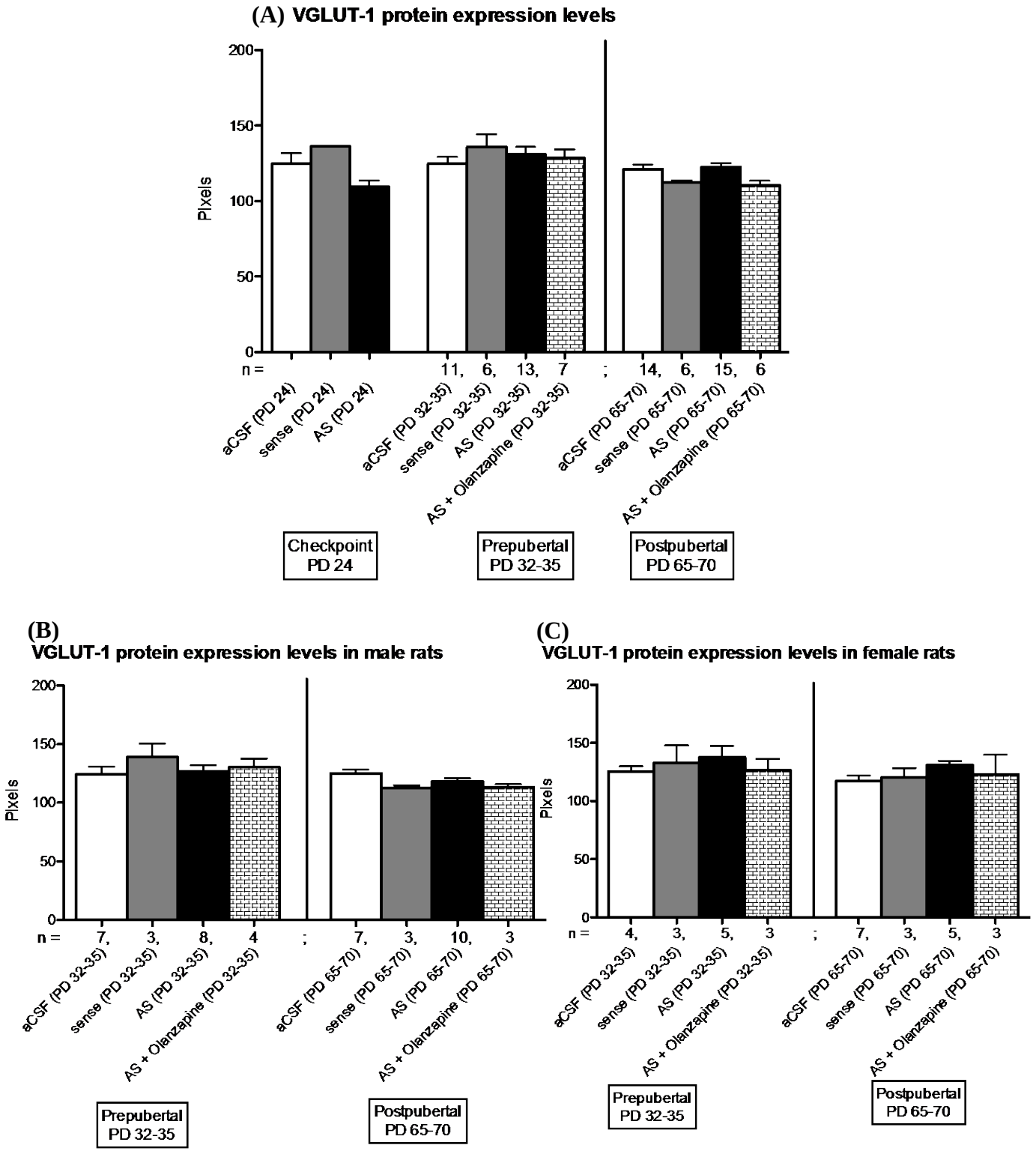

(B)
Synapsin II protein expression levels in male rats
Prepubertal Stage (PD 32-35)
Postpubertal Stage (PD 65-70)
Pixels
200
150
100
50
0
*
n = 7, 3, 8, 4
aCSF IIA
sense IIA
AS IIA
AS + Olanzapine IIA
aCSF IIB
sense IIB
AS IIB
AS + Olanzapine IIB
Synapsin IIa
Synapsin IIb


(C)
Synapsin II protein expression levels in female rats
Prepubertal Stage (PD 32-35)
Postpubertal Stage (PD 65-70)
Pixels
150
100
50
0
n = 4, 3, 5, 3
aCSF IIA
sense IIA
AS IIA
AS + Olanzapine IIA
aCSF IIB
sense IIB
AS IIB
AS + Olanzapine IIB
Synapsin IIa
Synapsin IIb

Figures 27A-C: Graphs indicate protein expression levels of synapsin isoforms IIa and IIb in the mPFC resulting from developmental synapsin II knockdown (PD 17-23). Immunoblotting assessed protein expression levels of synapsin IIa and IIb at both pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages. A significant decrease in synapsin IIa protein expression was found to persist only to pre-pubertal stage PD 32-35,of which deficit was attenuated with olanzapine treatment. There were no significant changes in synapsin IIb expression levels at both pre-pubertal and post-pubertal stages. (**A**) At PD 32-35: $F(2,26) = 8.547$. (**B**) At PD 32-35, $F(3,17) = 4.612$. (**C**) No significant trends were found in female rats. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty stage. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

4.4.2. Effect of PD 17-23 developmental knockdown on protein expression levels of VGLUT-1

Immunoblotting results revealed that synapsin II knockdown during PD 17-23 did not influence the protein expression levels of vesicular glutamate transporter, VGLUT-1, in the mPFC of the rat at PD 24. In addition, synapsin II knockdown during neurodevelopment stages PD 17-23 did not induce lasting changes in VGLUT-1 protein expression levels at both pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages (**Figure 28A**). In addition, there were no gender differences in the protein expression of VGLUT-1 in the mPFC at both pre-pubertal PD 32-35 and post-pubertal PD 65-70 stages (**Figures 28B-C**).

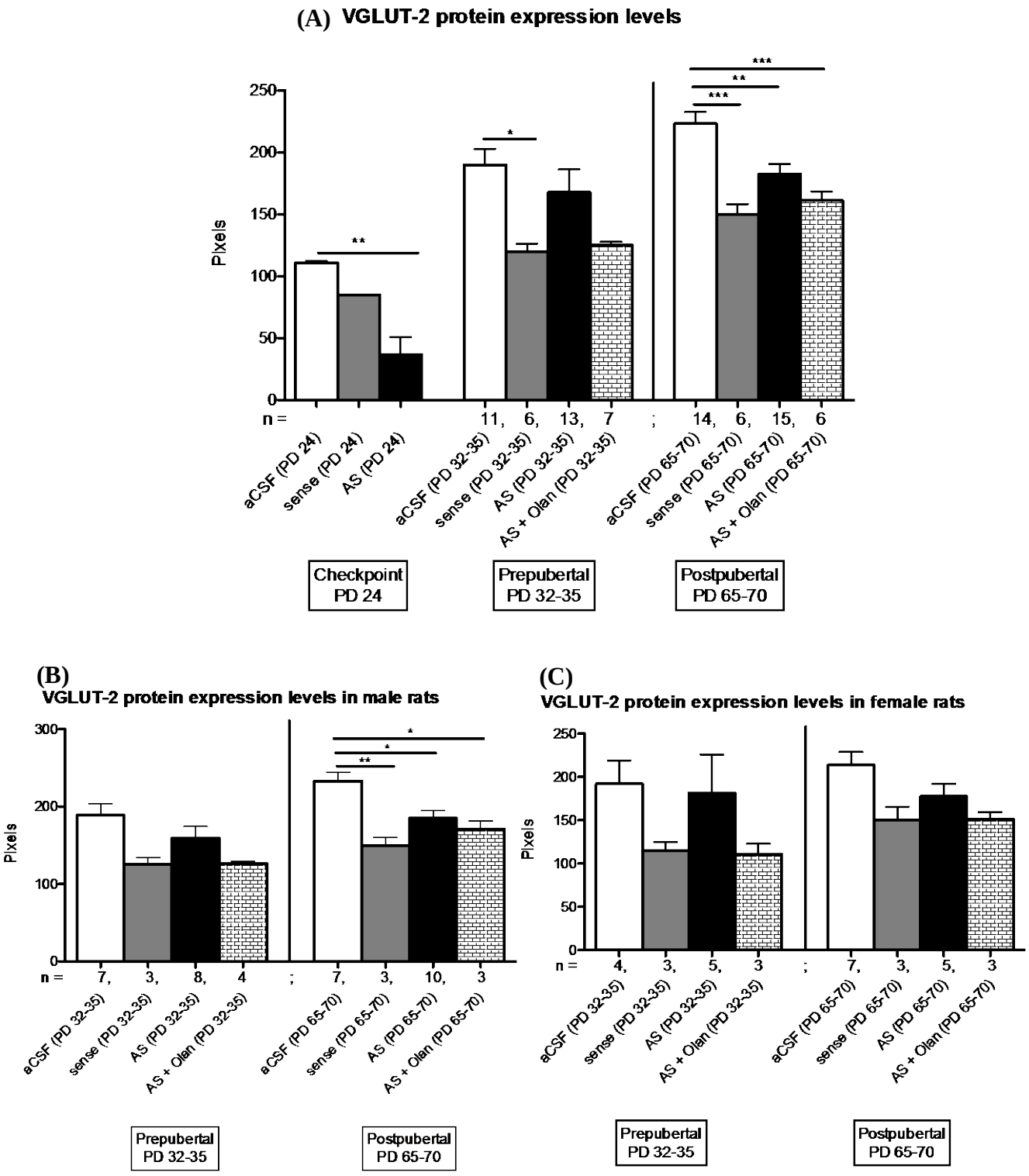

(A) VGLUT-1 protein expression levels
Pixels
200
150
100
50
0
n = 11, 6, 13, 7 ; 14, 6, 15, 6
aCSF (PD 24)
sense (PD 24)
AS (PD 24)
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olanzapine (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olanzapine (PD 65-70)
Checkpoint PD 24
Prepubertal PD 32-35
Postpubertal PD 65-70
(B)
VGLUT-1 protein expression levels in male rats
Pixels
n = 7, 3, 8, 4 ; 7, 3, 10, 3
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olanzapine (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olanzapine (PD 65-70)
Prepubertal PD 32-35
Postpubertal PD 65-70
(C)
VGLUT-1 protein expression levels in female rats
Pixels
n = 4, 3, 5, 3 ; 7, 3, 5, 3
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olanzapine (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olanzapine (PD 65-70)
Prepubertal PD 32-35
Postpubertal PD 65-70

Figures 28A-C: Graphs indicate protein expression levels of the vesicular glutamate transporter 1 (VGLUT-1) in the mPFC resulting from developmental synapsin II knockdown (PD 17-23). Immunoblotting assessed protein expression levels of VGLUT-2 both pre-pubertal (PD 24, PD 32-35) and post-pubertal (PD 65-70) stages. (**A**) There was no significant trend in VGLUT-1 expression levels at both pre-pubertal (PD 32-35) and post-pubertal stages (PD 65-70). (**B-C**) Gender differences did not affect the protein expression levels of VGLUT-1 in the mPFC at both pre-pubertal PD 32-35 and post-pubertal PD 65-70 stages. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar.

4.4.3. Effect of PD 17-23 developmental knockdown on protein expression levels of VGLUT-2

Immunoblotting results revealed that synapsin II knockdown during PD 17-23 induced a significant decrease in protein expression levels of vesicular transporter 2, VGLUT-2,: $F(3,8) = 4.312$ in the one-way analysis of variance, aCSF vs. AS $^{**}p < 0.01$ in post-hoc analysis immediately after the last day of infusion on PD 24. A reduction in VGLUT-2 protein expression levels was found in the sense treatment group as when compared to aCSF treatment: $F(3,32) = 3.971$ in the one-way analysis of variance, aCSF vs. sense $^{*}p < 0.05$. This neurodevleopmental decrease in VGLUT-2 protein expression levels was found to persist into post-pubertal stage PD 65-70 in sense-, AS- and AS + olanzapine-treated animals as compared to aCSF-treated controls. At PD 65-70, $F(3,37) = 0.4730$ in the one-way analysis of variance, aCSF vs sense $^{***}p < 0.001$, aCSF vs. AS $^{**}p < 0.01$, and aCSF vs. AS + olanzapine $^{***}p < 0.001$ in post-hoc analyses (**Figure 29A**).

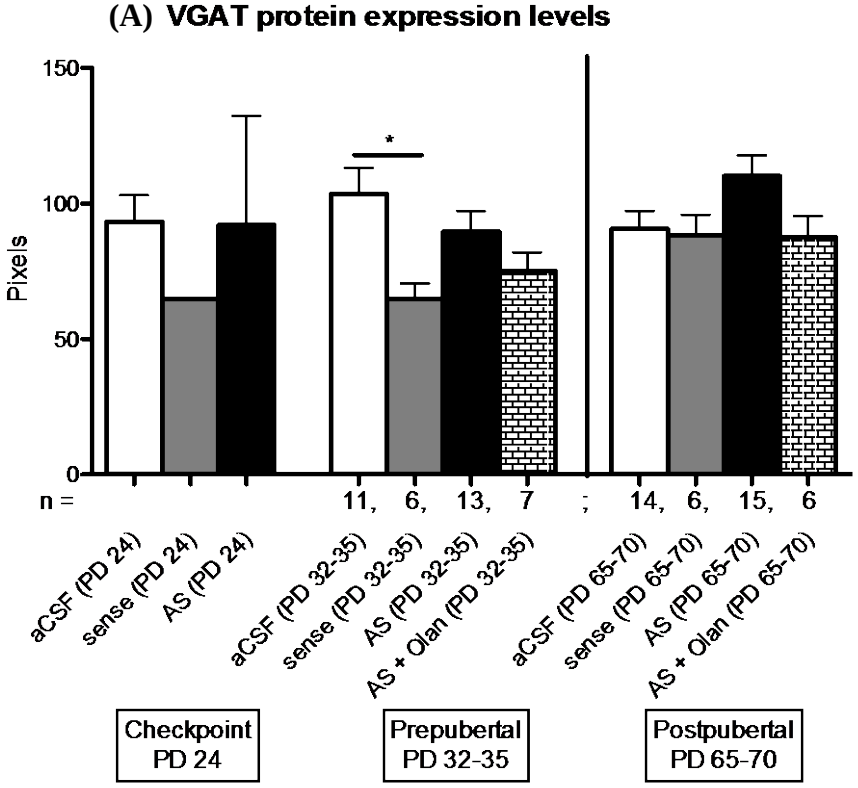
Gender differences were found in the protein expression of VGLUT-2 in the mPFC at the post-pubertal PD 65-70 stage. In male rats, a significant reduction in VGLUT-2 protein expression levels was found in sense-, AS-, and AS + olanzapine-treated animals as when compared to aCSF-treated animals. At PD 65-70, $F(3,19) = 7.220$ in the one-way analysis of variance, aCSF vs sense $^{**}p < 0.01$, aCSF vs. AS $^{*}p < 0.05$, and aCSF vs. AS + olanzapine $^{*}p < 0.05$ in post-hoc analyses of male rats (**Figure 29B**). Female rats showed similar trends in VGLUT-2 protein expression levels, but analyses did not reveal any significance between treatment groups (**Figure 29C**).

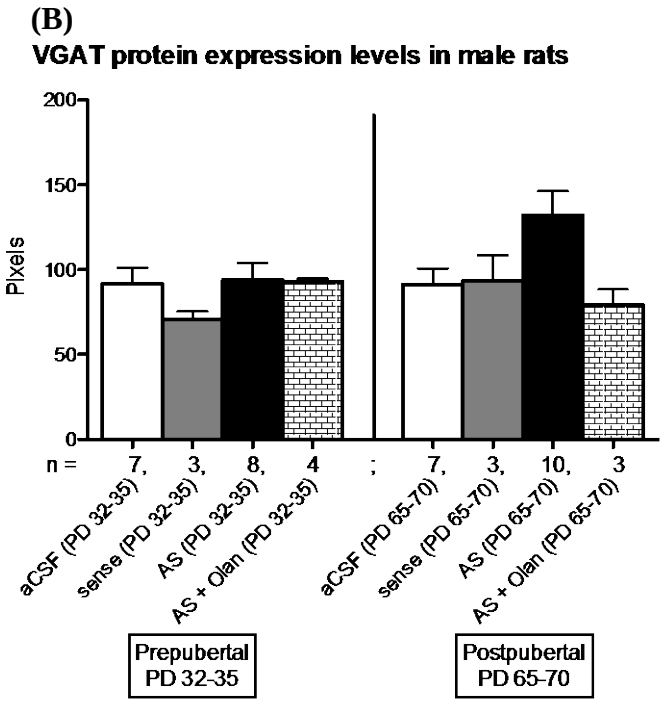

(A) VGLUT-2 protein expression levels
Pixels
250
200
150
100
50
0
**
*
***
**
***
n = 11, 6, 13, 7 ; 14, 6, 15, 6
aCSF (PD 24)
sense (PD 24)
AS (PD 24)
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olan (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olan (PD 65-70)
Checkpoint PD 24
Prepubertal PD 32-35
Postpubertal PD 65-70
(B)
VGLUT-2 protein expression levels in male rats
Pixels
300
200
100
0
*
*
**
n = 7, 3, 8, 4 ; 7, 3, 10, 3
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olan (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olan (PD 65-70)
Prepubertal PD 32-35
Postpubertal PD 65-70
(C)
VGLUT-2 protein expression levels in female rats
Pixels
250
200
150
100
50
0
n = 4, 3, 5, 3 ; 7, 3, 5, 3
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olan (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olan (PD 65-70)
Prepubertal PD 32-35
Postpubertal PD 65-70

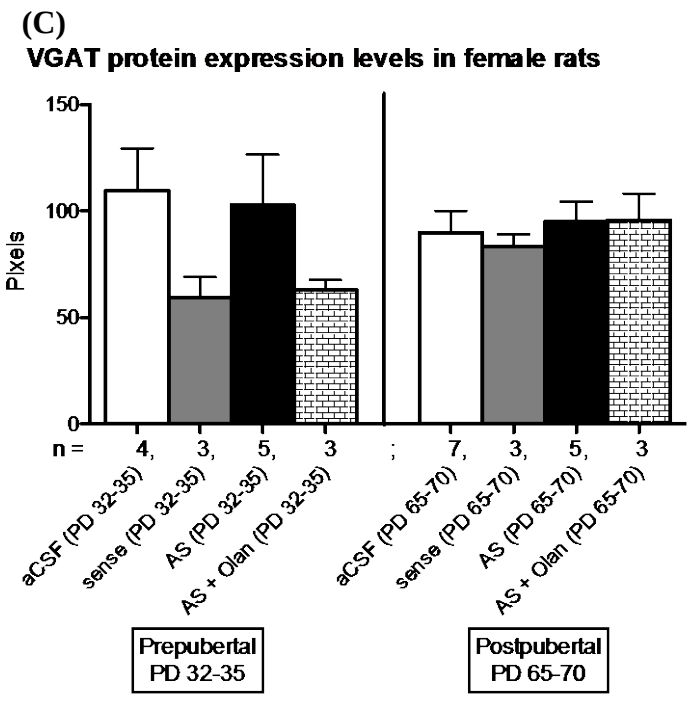
Figures 29A-C: Graphs indicate protein expression levels of the vesicular glutamate transporter 2 (VGLUT-2) in the mPFC resulting from developmental synapsin II knockdown (PD 17-23). Immunoblotting assessed protein expression levels of VGLUT-2 both pre-pubertal (PD 24, PD 32-35) and post-pubertal (PD 65-70) stages. (**A**) At PD 24: $F(3,8) = 4.312$. At PD 32-35, $F(3,32) = 3.971$. At PD 65-70, $F(3,37) = 0.4730$. (**B**) At PD 65-70, $F(3,19) = 7.220$. (**C**) No significant trends were found in female rats. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

4.4.4. Effect of PD 17-23 developmental knockdown on protein expression levels of VGAT

Immunoblotting results revealed that synapsin II knockdown during PD 17-23 did not influence the protein expression levels of VGAT in the mPFC of the rat at PD 24. There was a significant decrease in VGAT protein expression levels at pre-pubertal stage PD 32-35: $F(3,32) = 3.701$ in the one-way analysis of variance, aCSF vs. sense $*p < 0.05$ in post-hoc analysis. Synapsin II knockdown during neurodevelopmental stages PD 17-23 did not induce any other lasting changes in VGAT protein expression levels at both pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages (**Figure 30A**). In addition, there were no gender differences in the protein expression of VGAT in the mPFC at pre-pubertal PD 32-35 and post-pubertal PD 65-70 stages (**Figures 30B-C**).


(A) VGAT protein expression levels
Pixels
150
100
50
0
*
n = 11, 6, 13, 7 ; 14, 6, 15, 6
aCSF (PD 24)
sense (PD 24)
AS (PD 24)
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olan (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olan (PD 65-70)
Checkpoint PD 24
Prepubertal PD 32-35
Postpubertal PD 65-70


(B)
VGAT protein expression levels in male rats
Pixels
200
150
100
50
0
n = 7, 3, 8, 4 ; 7, 3, 10, 3
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olan (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olan (PD 65-70)
Prepubertal PD 32-35
Postpubertal PD 65-70

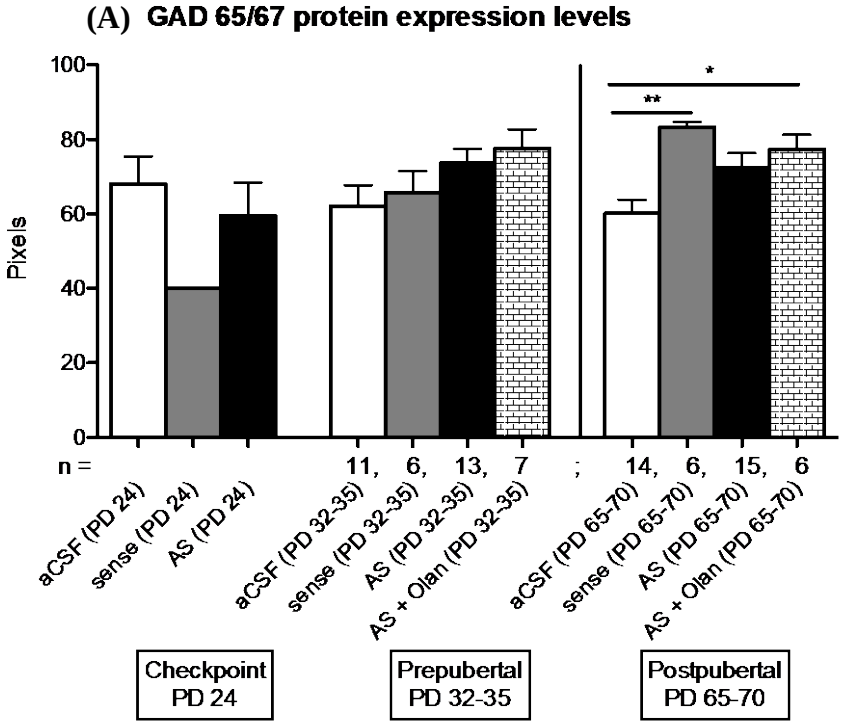

(C)
VGAT protein expression levels in female rats
Pixels
150
100
50
0
n = 4, 3, 5, 3 ; 7, 3, 5, 3
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olan (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olan (PD 65-70)
Prepubertal PD 32-35
Postpubertal PD 65-70

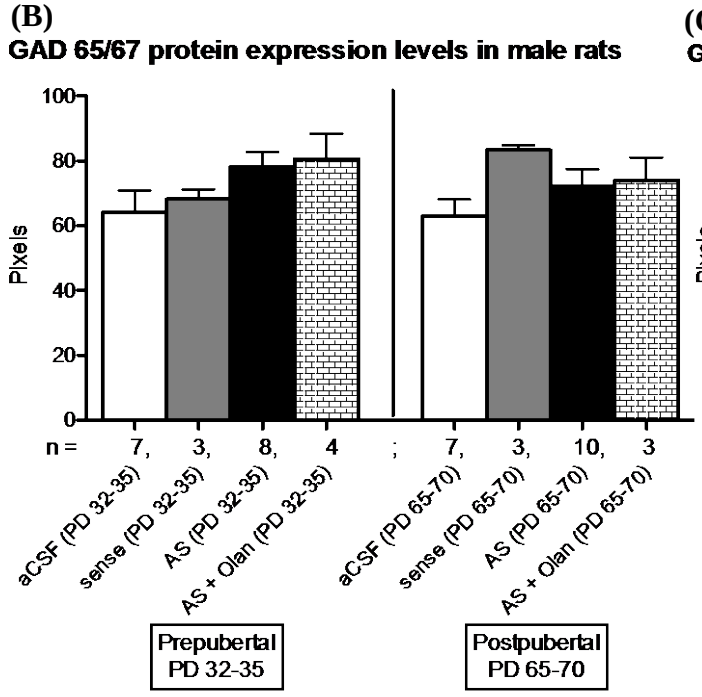
Figures 30A-C: Graphs indicate protein expression levels of VGAT in the mPFC resulting from developmental synapsin II knockdown (PD 17-23). Immunoblotting assessed protein expression levels of VGAT both pre-pubertal (PD 24, PD 32-35) and post-pubertal (PD 65-70) stages. At pre-pubertal stage PD 32-35, $F(3,32) = 3.701$, $*p < 0.05$. (**A**) No other significant trends were found in VGAT expression levels at both pre-pubertal (PD 32-35) and post-pubertal stages (PD 65-70). (**B-C**) Gender differences did not affect the protein expression levels of VGAT in the mPFC at both pre-pubertal PD 32-35 and post-pubertal PD 65-70 stages. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar.

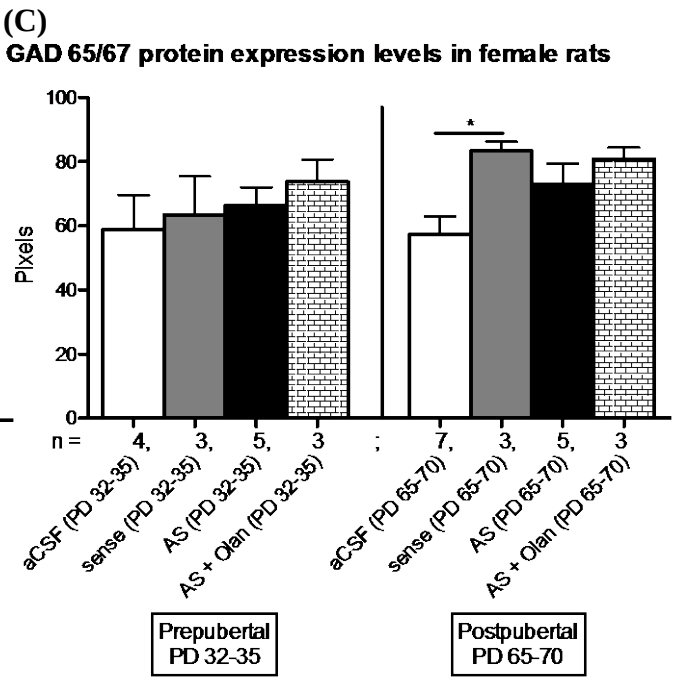
4.4.5. Effect of PD 17-23 developmental knockdown on protein expression levels of $GAD_{65/67}$

Immunoblotting results revealed that synapsin II knockdown during PD 17-23 did not significantly affect protein expression levels of $GAD_{65/67}$ in the mPFC of the rat at PD 24. There was a significant increase in $GAD_{65/67}$ protein expression levels which appeared at post-pubertal stage PD 65-70 in both sense- and AS + olanzapine- treated animals as when compared to aCSF treatment: $F(3,37) = 5.684$ in the one-way analysis of variance, aCSF vs. sense $^{**}p < 0.01$ and aCSF vs. AS + olanzpine $^{*}p < 0.05$ in post-hoc analyses (**Figure 31A**).

Both male and female rats displayed similar trends in $GAD_{65/67}$ protein expression levels (**Figures 31B-C**). However, the only significant trend was found in for the protein expression levels of $GAD_{65/67}$ at postpubertal PD 65-70 of female rats: $F(3,14) = 0.0229$ in the one-way analysis of variance, aCSF vs. sense $^{*}p < 0.05$ in post-hoc analysis (**Figure 31C**).


(A) GAD 65/67 protein expression levels
Pixels
100
80
60
40
20
0
*
**
n = 11, 6, 13, 7 ; 14, 6, 15, 6
aCSF (PD 24)
sense (PD 24)
AS (PD 24)
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olan (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olan (PD 65-70)
Checkpoint PD 24
Prepubertal PD 32-35
Postpubertal PD 65-70

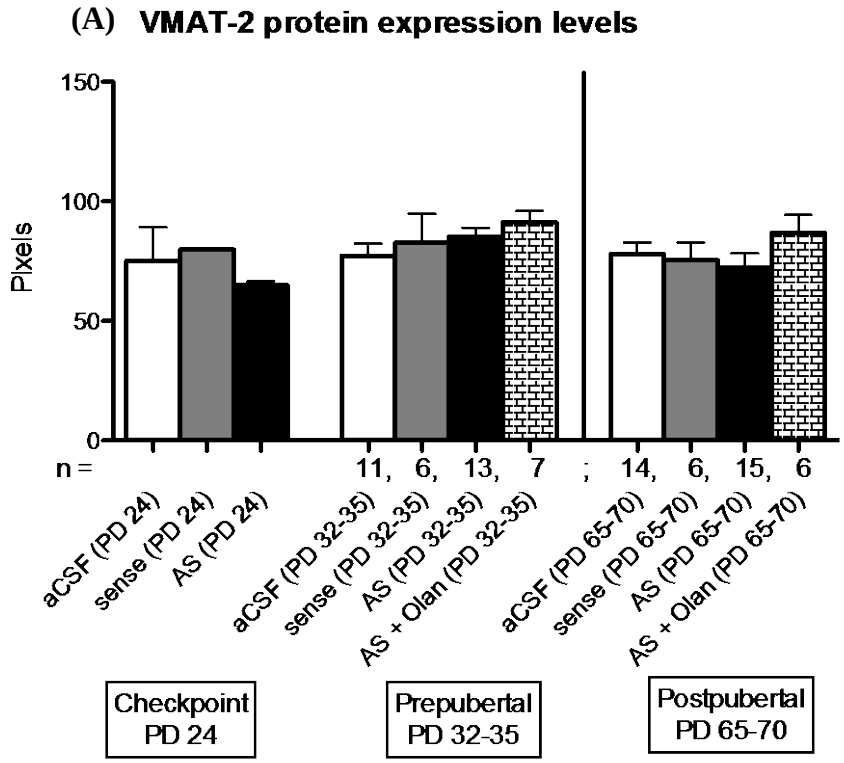

(B)
GAD 65/67 protein expression levels in male rats
Pixels
100
80
60
40
20
0
n = 7, 3, 8, 4 ; 7, 3, 10, 3
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olan (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olan (PD 65-70)
Prepubertal PD 32-35
Postpubertal PD 65-70

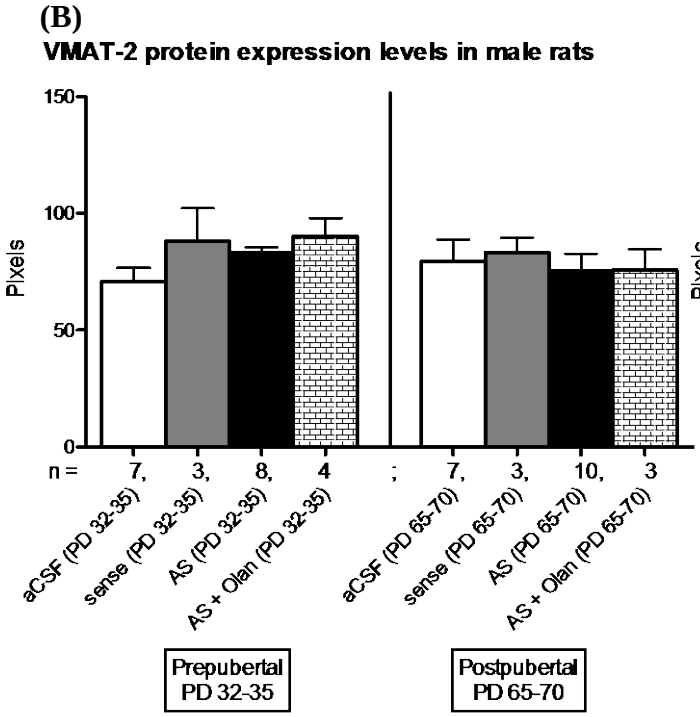

(C)
GAD 65/67 protein expression levels in female rats
Pixels
100
80
60
40
20
0
*
n = 4, 3, 5, 3 ; 7, 3, 5, 3
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olan (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olan (PD 65-70)
Prepubertal PD 32-35
Postpubertal PD 65-70

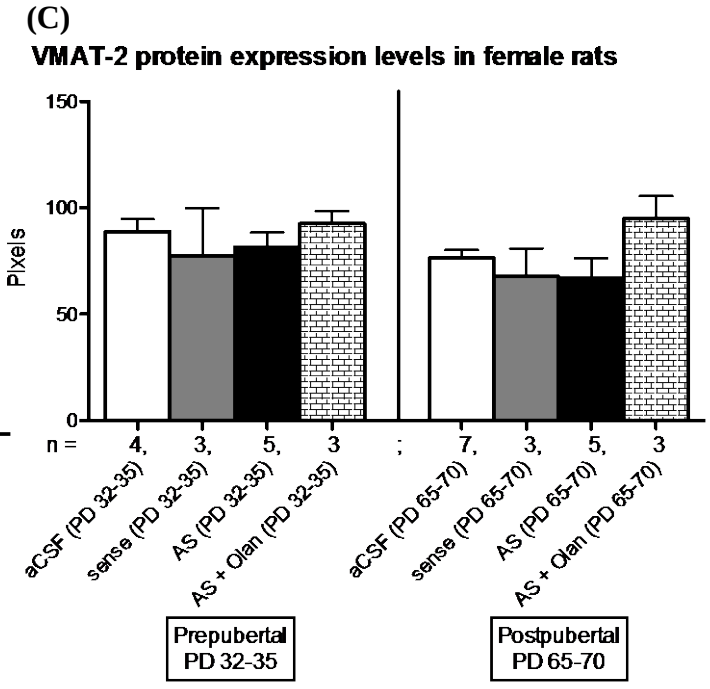
Figures 31A-C: Graphs indicate protein expression levels of $GAD_{65/67}$ in the mPFC resulting from developmental synapsin II knockdown (PD 17-23). Immunoblotting assessed protein expression levels of $GAD_{65/67}$ at both pre-pubertal (PD 24, PD 32-35) and post-pubertal (PD 65-70) stages. (**A**) At PD 65-70, $F(3,37) = 5.684$. (**B**) No significant trends were found in male rats (**C**) At PD 65-70, $F(3,14) = 0.0229$. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar. $*p < 0.05$, $**p < 0.01$.

4.4.6. Effect of PD 17-23 developmental knockdown on protein expression levels of VMAT-2

Immunoblotting results revealed that synapsin II knockdown during PD 17-23 did not influence the protein expression levels of VMAT-2 in the mPFC of the rat at PD 24. In addition, synapsin II knockdown during neurodevelopmental stages PD 17-23 did not induce lasting changes in VMAT-2 protein expression levels at both pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages (**Figure 32A**). In addition, there were no gender differences in the protein expression of VMAT-2 in the mPFC at both pre-pubertal PD 32-35 and post-pubertal PD 65-70 stages (**Figures 32B-C**).


(A) VMAT-2 protein expression levels
Pixels
150
100
50
0
n = 11, 6, 13, 7 ; 14, 6, 15, 6
aCSF (PD 24)
sense (PD 24)
AS (PD 24)
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olan (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olan (PD 65-70)
Checkpoint PD 24
Prepubertal PD 32-35
Postpubertal PD 65-70

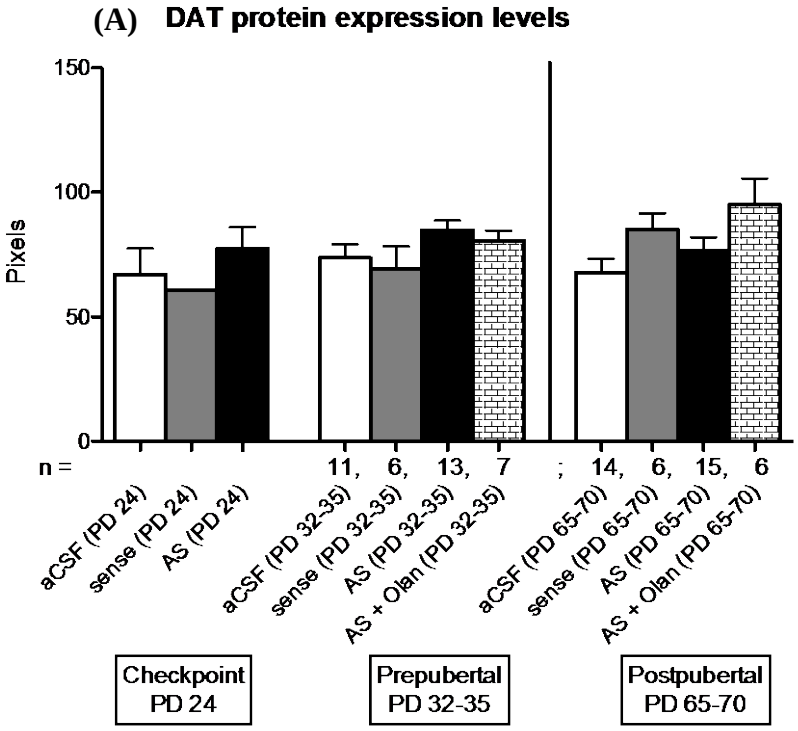

(B)
VMAT-2 protein expression levels in male rats
Pixels
150
100
50
0
n = 7, 3, 8, 4 ; 7, 3, 10, 3
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olan (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olan (PD 65-70)
Prepubertal PD 32-35
Postpubertal PD 65-70

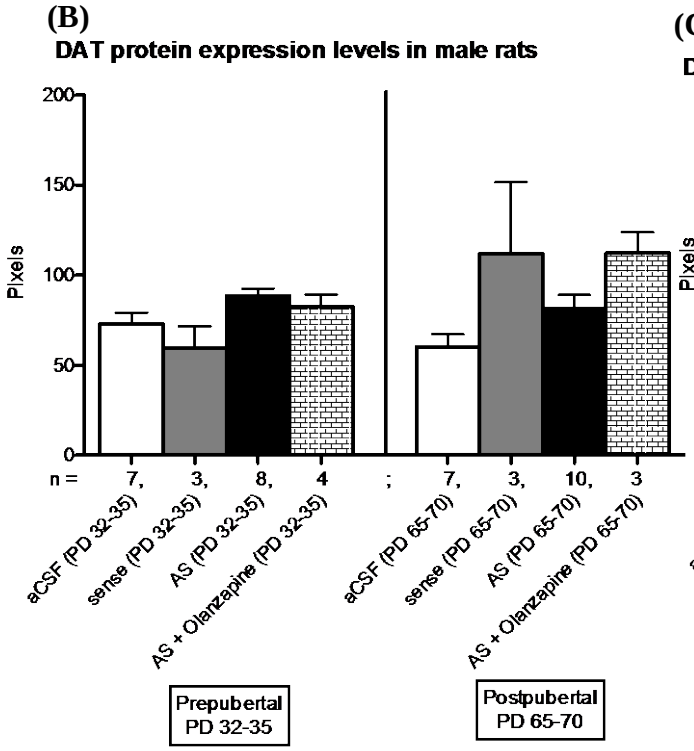

(C)
VMAT-2 protein expression levels in female rats
Pixels
150
100
50
0
n = 4, 3, 5, 3 ; 7, 3, 5, 3
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olan (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olan (PD 65-70)
Prepubertal PD 32-35
Postpubertal PD 65-70

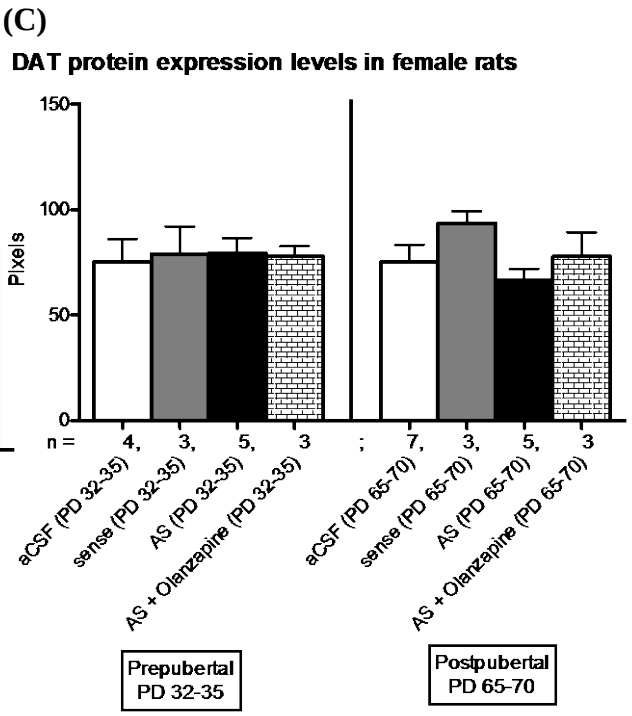
Figures 32A-C: Graph indicate protein expression levels of the VMAT-2 in the mPFC resulting from developmental synapsin II knockdown (PD 17-23). Immunoblotting assessed protein expression levels of VMAT-2 both pre-pubertal (PD 24, PD 32-35) and post-pubertal (PD 65-70) stages. (**A**) There was no significant trend in VMAT-2 expression levels at both pre-pubertal (PD 32-35) and post-pubertal stages (PD 65-70). (**B-C**) Gender differences did not affect the protein expression levels of VMAT-2 in the mPFC at both pre-pubertal PD 32-35 and post-pubertal PD 65-70 stages. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar.

4.4.7. Effect of PD 17-23 developmental knockdown on protein expression levels of DAT

Immunoblotting results revealed that synapsin II knockdown during PD 17-23 did not influence the protein expression levels of DAT in the mPFC of the rat at PD 24. In addition, synapsin II knockdown during neurodevelopmental stages PD 17-23 did not induce lasting changes in DAT protein expression levels at both pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages (**Figure 33A**). In addition, there were no gender differences in the protein expression of DAT in the mPFC at both pre-pubertal PD 32-35 and post-pubertal PD 65-70 stages (**Figures 33B-C**).


(A) DAT protein expression levels
Pixels
150
100
50
0
n = 11, 6, 13, 7 ; 14, 6, 15, 6
aCSF (PD 24)
sense (PD 24)
AS (PD 24)
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olan (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olan (PD 65-70)
Checkpoint PD 24
Prepubertal PD 32-35
Postpubertal PD 65-70


(B)
DAT protein expression levels in male rats
Pixels
200
150
100
50
0
n = 7, 3, 8, 4 ; 7, 3, 10, 3
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olanzapine (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olanzapine (PD 65-70)
Prepubertal PD 32-35
Postpubertal PD 65-70


(C)
DAT protein expression levels in female rats
Pixels
150
100
50
0
n = 4, 3, 5, 3 ; 7, 3, 5, 3
aCSF (PD 32-35)
sense (PD 32-35)
AS (PD 32-35)
AS + Olanzapine (PD 32-35)
aCSF (PD 65-70)
sense (PD 65-70)
AS (PD 65-70)
AS + Olanzapine (PD 65-70)
Prepubertal PD 32-35
Postpubertal PD 65-70

Figures 33A-C: Graph indicate protein expression levels of the DAT in the mPFC resulting from developmental synapsin II knockdown (PD 17-23). Immunoblotting assessed protein expression levels of DAT both pre-pubertal (PD 24, PD 32-35) and post-pubertal (PD 65-70) stages. (**A**) There was no significant trend in DAT expression levels at both pre-pubertal (PD 32-35) and post-pubertal stages (PD 65-70). (**B-C**) Gender differences did not affect the protein expression levels of DAT in the mPFC at both pre-pubertal PD 32-35 and post-pubertal PD 65-70 stages. Vertical line is drawn between pre-pubertal and post-pubertal stages to signify puberty stage. aCSF-treated group is indicated by white bars, sense-treated group is indicated by grey bars, AS-treated group is indicated by black bar, and AS + olanzapine treated group is indicated by brick-patterned bar.

5. DISCUSSION

5.1 Synapsin II knockdown during PD 7 is insufficient to induce lasting schizophrenia-like behavioural abnormalities into adulthood.

Brain development is dynamic process. The mammalian brain first undergoes a period of overproduction of synapses and receptors, which is then followed secondly by an aggressive elimination of synapses immediately before birth and during transition stages in adulthood (Andersen, 2003). This framework creates windows of vulnerability in which interruptions can deter the normal process of brain development and promote the production of mental illnesses. In order for the developing brain to be vulnerable, both the insulting agent and the vulnerable period of exposure must be determined. Studies from our laboratory have previously established a decrease in synapsin II expression to correlate with the production of schizophrenia-like behaviour, both in animals and in patients with schizophrenia. Seeing as the vulnerable gene has been established, we sought to determine the critical period of insult in this study.

With reference to the expression levels of synapsin II in the developing brain, a knockdown of synapsin II was targeted at both the initiation and maximal expression periods (**Figure 8**). A one-time knockdown during the initiation period of synapsin II expression on PD 7 was found to be insufficient at inducing lasting schizophrenia-like changes in adulthood. Behavioural assessments during both pre-pubertal (PD 32-35) and post-pubertal (PD 65-70, PD 85-90, PD 122) stages did not indicate the presence of schizophrenia-like behavioural abnormalities (i.e. no increase in locomotor activity, no decrease in social interaction, no deficit in prepulse inhibition) (**Figures 10A-B, 11A-D,**

12A-C). This lack of an influence from a one-time PD 7 synapsin II knockdown could easily be due to the compensation of synapsin II expression levels immediately following this subtle knockdown period (PD 7) (**Figure 8**). Moreover, following the two-stage process of brain development previously mentioned, an interruption of synapsin II expression levels at the initiation period of synapsin II expression may be less influential than an interruption of these same synapsin II expression levels during the maximal expression period (i.e. interruption during the period of overproduction and agressive elimination of synapsin II). Accordingly, the next stage of the study was to examine the effects of synapsin II knockdown during the maximal period of synapsin II expression.

5.2. Synapsin II knockdown during PD 17-23 induces lasting schizophrenia-like behavioural changes into adulthood which are attenuated with olanzapine treatment

Synapsin II knockdown during neurodevelopmental period PD 17-23 was found to induce lasting schizophrenia-like behavioural changes into adulthood. These behavioural abnormalities were measured both at pre-pubertal (PD 32-35) and post-pubertal (PD 65-70, PD 55-59, PD 65-70, PD 86-90, PD 168-169) stages. Unlike the one-time PD 7 knockdown, the lasting influence of a knockdown during PD 17-23 may be due to a lack of compensation following this period of synapsin II over-production (PD 17-23) (**Figure 8**). Evidence suggest that PD 17-23 may be a critical period for synapsin II expression. Interestingly, this period of brain development in the rat parallels the third trimester of pregnancy in humans (Clancy, Darlington, & Finlay, 2001). This suggests that the third trimester of pregnancy may be a period of vulnerability in humans, whereby

the uninterrupted development of synapses in the PFC is critical. A genetic predisposition directly or indirectly influencing suboptimal expression of synapsin II levels in the PFC at this stage may negatively influence brain development and increase susceptibility to developing schizophrenia.

Although much smaller and less developed in rodents, the mPFC of the rat is thought to be analogous to the dorsolateral PFC in humans, the region of the PFC indicated by decades of research to be most affected in schizophrenia (DeVito, Lykken, Kanter, & Eichenbaum, 2010; Guillozet-Bongaarts et al., 2014). Both the mPFC in the rodent and dorsolateral PFC in the primate show similar projections from the thalamus (DeVito et al., 2010). Moreover, these two regions have been shown in studies to be functionally similar and involved in comparable brain functions such as the organization and integration of episodic memory, as well as other "executive functions" such as decision-making, planning, attentional selection, behavioural inhibition, and task switching (DeVito et al., 2010).

In our study, a temporal synapsin II knockdown during PD 17-23 in the mPFC of the rat induced locomotor hyperactivity (**Figures 16A-B, 17A-B, 18A-B, 19A-B**), social withdrawal (**Figures 20A-D, 21A-D, 22A-D, 23A-D**), and deficits in prepulse inhibition (**Figures 24A-C, 25A-F, 26A-C**). These schizophrenia-like behavioural abnormalities were present at pre-pubertal (PD 32-35) and persisted into post-pubertal (PD 55-59, PD 65-70, PD 85-90, PD 168-169) stages. The production of these behavioural abnormalities provide evidence for the face validity of this animal model of schizophrenia, and will be described in further detail in sections 5.2.1 - 5.2.3.

Predictive validity has also been demonstrated in this newly developed neurodevelopmental model of schizophrenia. Treatment with atypical antipsychotic drug, olanzapine, was found to effectively attenuate the manifested abnormalities related to schizophrenia. Acute treatment with olanzapine effectively attenuated locomotor hyperactivity (**Figures 16A-B, 17A-B, 18A-B, 19A-B**), social withdrawal (**Figures 20A-D, 21A-D, 22A-D, 23A-D**), and deficits in prepulse inhibition (**Figures 24A-C, 25A-F, 26A-C**) at pre-pubertal and post-pubertal stages of the developmental synapsin II knockdown animals. Previous studies from our laboratory testing the efficacy antipsychotic drugs in the adult synapsin II knockdown model indicated that only atypical, but not typical, classes of drugs were effective at intervening the hypoglutamatergic deficits associated with synapsin II knockdown (Dyck et al., 2011). Further studies must assess the effectiveness of other classes of antipsychotic drugs in order to strengthen the predictive validity of this novel neurodevelopmental animal model of schizophrenia.

Adolescence is a period marked by sudden hormonal changes and brain maturation, which can significantly influence behaviour during this period. Clinical onset of schizophrenia occurs during the periadolescence period, as such, either or both processes of hormonal changes and brain maturation during this period can contribute to symptom manifestation in schizophrenia (Andersen, 2003). Adolescence is viewed as a key maturational period of processes in the brain and body, and pubertal increase in gonadal hormones are a hallmark of changes during this period (Spear, 2000). Some studies suggest that an increase in stress cortisol and pronounced activation of the

hypothalamic-pituitary-adrenal axis during the period of adolescence triggers the expression of genes involved in adolescent brain development and neuron survival (Walker et al., 2002). Gonadal and adrenal hormones have been suggested to have both activational and organization effects on brain development and on the production of sexual, agonistic, and anxiety-related behaviours (Walker et al., 2002; Sisk & Zehr, 2005). In addition to hormonal fluctuations, the major developmental processes of the PFC have also been observed to occur during this period of adolescence across species (**Figure 8**) (Spear, 2000). Thus, other researchers suggest that the manifestation of schizophrenia revolves around the development of affected brain regions (i.e. PFC) as opposed to hormonal changes during adolescence. In line with this, a study by Lipska *et al.* displayed that the observed changes during adolescence in the NVHL model was independent of hormonal surge and fluctuations, since induced schizophrenia-like behavioural abnormalities were still observed in animals depleted of gonadal hormones before puberty (Lipska et al., 1994; Tseng et al., 2009; Lipska, 2004). Thus, gonadal hormones could potentially have no effect on symptom manifestation in schizophrenia.

In our neurodevelopmental synapsin II knockdown animal model of schizophrenia, behavioural abnormalities were present at both pre-pubertal and post-pubertal stages of development. The maturation and synaptic pruning of our target brain region of interest, the mPFC, occurs from PD 30 onwards in the rat (**Figure 1**). Maturation of the mPFC initiates pre-puberty and symptom manifestation similarly develops at pre-pubertal stages (PD 32-35), after the initiation of mPFC pruning (PD 30, **Figure 1**) and prior drastic changes in pubertal hormones (puberty: PD 35-55, **Figure 9**).

Moreover, similar behavioural trends between treatment groups were observed in both the male and female population of animals, disregarding a significant sway of hormonal differences in synapsin II knockdown-induced symptom production. If hormonal changes during adolescence has an effect on behaviour development, symptoms at post-pubertal stages should be observed with greater severity than symptoms (if any) observed at pre-pubertal stages. Altogether, evidence suggest that the presentation of schizophrenia-like symptoms likely revolves around the maturation of the mPFC and is independent of hormonal fluctuations. Further studies must be performed to definitively exclude the contribution of hormonal changes during puberty in symptom manifestation within this neurodevelopmental animal model of schizophrenia. Changes in clinical schizophrenia could very well be attributed to both brain development and hormonal changes, and neither theories have been exhaustively excluded as factors influencing clinical outcome.

5.2.1. Synapsin II knockdown during PD 17-23 induces locomotor hyperactivity which persist into pre-pubertal and post-pubertal stages of adulthood.

The measurement of locomotor activity has been widely used to model the positive symptoms of schizophrenia and is a reliable predictor of psychosis in animal models of the disease (van den, Garner, Gogos, & Kusljic, 2005). Early studies have shown the importance of increased dopamine levels within the ventral striatum/NAc in the production of amphetamine-induced or PCP-induced hyperactivity (van den et al., 2005). This spike in dopamine activity has been similarly found in patients with schizophrenia challenged with amphetamine (van den et al., 2005). Striatal dopamine

increase in the human brain induces the production of euphoria and psychosis in addition to hyperactivity, while the same striatal dopamine increase in the rodent brain induces locomotor hyperactivity (van den et al., 2005). This amphetamine-induced neurochemical similarity between animal models of schizophrenia and clinical patients provides evidence for the valid use of this behavioural assessment. When using animal models to study diseases, it is important to look for behavioural effects relevant to the species. Use of this behavioural assessment for the positive symptoms of schizophrenia has been further supported with typical and atypical antipsychotic drug validation studies (Ellenbroek & Cools, 2000). Treatment with antipsychotic drugs have reliably inhibited this increase in locomotor activity in animal models of schizophrenia, as they do with treatment of psychosis in patients with schizophrenia.

5.2.2. Synapsin II knockdown during PD 17-23 induces social withdrawal which persist into pre-pubertal and post-pubertal stages of adulthood.

In patients with schizophrenia, reductions in social behaviour presents itself in the premorbid stage of the disease (i.e. early pre-schizophrenic childhood), and generally persists thoughout the entire course of the disease. Although easily detected, social withdrawal, is one of the toughest measures to assess in humans given the active avoidance of patients towards social behaviour (Ellenbroek et al., 2000). Contrary to studying social withdrawal in patients with schizophrenia, however, social interaction is relatively easy to measure in animals and is a reliable predictor of the negative symptoms of schizophrenia (van den et al., 2005). Animals tend to have a stable degree of social

behaviour, which helps simplifies this behavioural analysis (Ellenbroek et al., 2000). Animal models of the disease, such as the pharmacological amphetamine- and PCP-induced or the neurodevelopmental NVHL animals, consistently display deficits in social behaviour. Moreover, treatment with antipsychotic drugs effectively attenuate this deficit in social interaction in animal models of schizophrenia (Ellenbroek et al., 2000; Lipska & Weinberger, 2000).

Brain regions responsible for social cognition include the mPFC, PFC, amydala, and inferior parietal lobe (Brunet-Gouet & Decety, 2006). Deficits in social functioning in schizophrenia have been suggested to result from abnormal interaction between the frontal lobe and the functionally connected cortical and subcortical regions of the brain (Lee, Farrow, Spence, & Woodruff, 2004). Some studies suggest that the negative symptoms of schizophrenia correlate in severity with the degree of functional and structural brain changes, including reduced gray matter volume in the temporal lobe and amydala, or grey and white matter loss in the mPFC and temporal lobes (Stahl & Buckley, 2007). Other studies found reduced blood flow into the temporal lobe and PFC of patients who predominantly possess the negative symptoms of schizophrenia (Stahl et al., 2007). Given the complexity surrounding social behaviour, it is difficult to pin-point the specific circuits mediating social deficits in schizophrenia (Lee et al., 2004; Brunet-Gouet et al., 2006). However, given the crucial role of synapsin II in the maintenance of synapse functioning, a depletion of this protein could conceivably disrupt local cortico-cortical and cortical-subcortical signalling, leading to the production of social withdrawal in the neurodevelopmental synapsin II knockdown animal.

5.2.3. Synapsin II knockdown during PD 17-23 induces deficits in prepulse inhibition which persist into pre-pubertal and post-pubertal stages of adulthood.

Prepulse inhibition has been widely used as a cross-species measure of sensorimotor gating, or the ability to "gate out" unnecessary stimuli (Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001; Braff, Geyer, & Swerdlow, 2001b). The test of prepulse inhibition is similarly performed in animals as it is in humans (van den et al., 2005). This task utilizes a simple phenomenon of sensory processing and tests the natural and unlearned gating mechanism to inhibit the presented acoustic startle (van den et al., 2005). Startle is mediated by neuronal circuits within limbic and cortical regions, including the mPFC, NAc, hippocampus, and amydala (van den et al., 2005). Disruptions in prepulse inhibition, or the inability to "gate out" unnecessary stimuli, is a consistent finding in both patients with schizophrenia and animal models of the disease (van den et al., 2005). This inhibition has been found to be associated with cognitive and attentional abnormalities; a loss of normal inhibition is thought to underlie sensory flooding and cognitive fragmentation in patients with schizophrenia (Geyer et al., 2001; Braff et al., 2001b).

Disruptions of prepulse inhibition can be induced by dopamine D_2 receptor stimulation, serotonin (5-HT) receptor agonism, and N-methyl-D-aspartate (NMDA) receptor antagonism. This assessment model has accordingly been used to identify potential therapeutics for schizophrenia (Geyer et al., 2001). Deficits in prepulse inhibition has been observed in our synapsin II neurodevelopmental animal model of schizophrenia. PPI deficits may result from hypoglutamatergic function in the PFC

stemming from synapsin II aberrations, or from dysregulated modulation of the dopamine D_2 receptors implicated in synapsin II regulation (Geyer et al., 2001; Chong et al., 2002; Chong et al., 2006). A developmental interruption of synapsin II was found to induce lasting deficits in prepulse inhibition, signifying the salience of this neurodevelopmental alteration to cause lasting brain circuitry alterations typical to clinical schizophrenia.

5.3. Synapsin II knockdown during PD 17-23 induces lasting schizophrenia-like neurochemical changes into adulthood.

Developmental knockdown of synapsin II during a critical period induced a loss of function in glutamatergic prefrontal cortical signalling, which has been seen to cause permanent changes to the neural circuits mediating dopamine- and glutamate- related behaviours in our animal model of schizophrenia. Partial construct validity is presented in this neonatal animal model of schizophrenia. A physical insult and knockdown of synapsin II is unlikely to occur in the developing brain of a human during the third trimester of pregnancy. Rather, a genetic predisposition is likely to influence the suboptimal expression of synapsin II at this perinatal period of development. Other environmental factors which contribute to the hypofunction of the PFC during this perinatal period can further aggravate prefrontal cortical development and contribute to disease development.

5.3.1. Synapsin II knockdown during PD 17-23 induces persistent alterations in the glutamate neurotransmission of the medial prefrontal cortex.

Following a temporal synapsin II knockdown during PD 17-23, synapsin II protein expression levels were further assessed at pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages. Interestingly, deficits in protein expression levels of synapsin IIa were maintained until pre-pubertal stages (PD 32-35) (**Figures 27A-C**). This deficit in synapsin IIa protein expression levels did not persist into post-pubertal stages (PD 65-70). Protein expression levels of synapsin IIb was normalized at both pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages (**Figures 27A-C**). Three things are worth noting: (1) Synapsin IIa is the functionally relevant isoform with its ability to rescue the function of glutamatergic neurons in a synapsin triple knockout animal (section 1.6.5.). Synapsin IIb was not found to have this same robust capability (Gitler et al., 2008). Thus, it is intuitive that only deficits in synapsin IIa, and not IIb, was sufficient to maintain the hypoglutamatergic state of the animal model and its associated schizophrenia-like behavioural deficits at prepubertal stages (PD 32-35) (**Figures 27A-C, 34A**). (2) The reduction in synapsin II expression was found to persist an additional 9-12 days past the final day of AS-infusion. Lasting molecular changes in synapsin II expression levels were found owing to a temporal synapsin II knockdown during the critical period of synapsin II expression (PD 17-23). Our new neurodevelopmental animal model of schizophrenia presents lasting changes which were not previously found in the adult synapsin II knockdown animal model. Expression levels of synapsin II in the mPFC of the previous adult synapsin II knockdown model was highly dependent on the presence of the AS sequence, such that synapsin II expression levels returned to baseline levels upon cessation of AS infusion. (3) Acute olanzapine treatment effectively attenuated the

decrease in synapsin IIa protein expression levels at pre-pubertal stage (PD 32-35) (**Figures 27A-C**). Olanzapine may increase synapsin II expression levels though its interactions with the dopamine receptors; previous studies from our laboratory indicate the involvement of dopamine D_1 and D_2 receptors in the gene regulation and synthesis of synapsin II (Chong et al., 2002; Chong et al., 2006). Antagonism of dopamine D_2 receptors within the densely D_2-populated striatum may also cause subsequent increase in glutamate signalling back into the cortical regions of the brain, thereby alleviating the hypoglutamatergic state of the PFC and attenuating the associated schizophrenia-like behaviours (**Figure 4B, 34A**). In addition, the affinity of olanzapine for serotonin 5-HT_{2A} receptors located on cortical GABA and glutamate neurons may further provoke an increase in synapsin IIa expression levels. Hypothesized mechanisms are described in detail in section 5.3.2.1.

Early developmental insult of synapsin II expression levels in the mPFC was found to facilitate the maladaptive development of the glutamate system leading to the variety of schizophrenia-related behavioural abnormalities assessed in adulthood. Glutamate has been found to promote various aspects of neuron development, including cell survival, migration, and differentiation (Mouri et al., 2007). The NMDA receptor is also implicated in neuron plasticity and the control of structure in the developing brain circuitry (Mouri et al., 2007). Various studies have looked into the suppression of glutamate function during development (i.e. PCP administration at perinatal stages) to induce permanent glutamate hypofunction related to schizophrenia (Mouri et al., 2007; Jones et al., 2011). Synapsin IIa, in particular, has been found to be directly associated

with glutamate-containing vesicles (Gitler et al., 2008). A suppression in expression levels of synapsin II in the PFC at during crucial stages of development would induce a suppression of glutamate activity, and thus stunt the growth, differentiation, and survival of prefrontal cortical neurons. This would induce abnormal signalling interactions in both the local and neighboring regions of the brain, and cause subsequent behavioural abnormalities arising from aberrant brain development and maturation (**Figure 34A-B**). Our findings further indicate that an increase in synapsin IIa may alleviate hypofunction within the glutamate system and sufficiently salvage the function of the PFC as well as attenuate schizophrenia-related behavioural abnormalities.

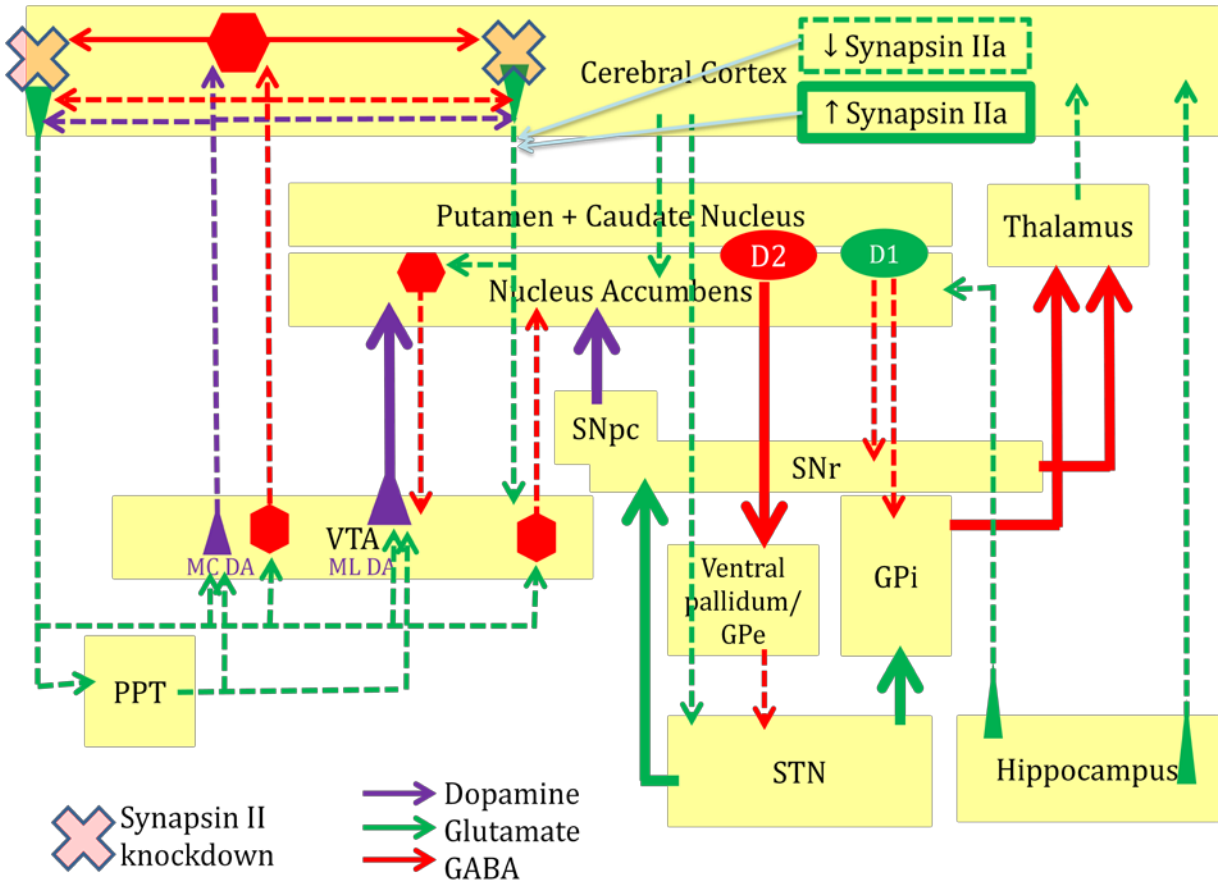


Figure 34A: Proposed maintenance of prefrontal cortical hypoglutamatergic state at pre-pubertal stages (PD 32-35). A temporal developmental knockdown of synapsin II (PD 17-23) resulted in lasting reductions in synapsin IIa protein expression levels in the mPFC at pre-pubertal stages (PD 32-35); this decrease in synapsin IIa protein expression is proposed to maintain the hypoglutamatergic state of the mPFC at this stage of development. Alleviation of hypoglutamatergic state in the mPFC is seen with an olanzapine treatment-induced increase in synapsin IIa protein expression at pre-pubertal stage (PD 32-35). Light blue arrows indicate the neuron populations in which assessed proteins of interest are hypothesized to act on.

Vesicular glutamate transporters, VGLUT-1 and VGLUT-2, are responsible for the trafficking of glutamate into synaptic vesicles and the exocytotic release of glutamate in established excitatory neurons (Fremeau, Jr., Voglmaier, Seal, & Edwards, 2004). VGLUT-1 and VGLUT-2, show complementary distribution in the brain, suggesting specialized roles for each vesicular transporter (Fremeau, Jr. et al., 2004). VGLUT-1 mRNA is strongly expressed in the cerebral cortex, hippocampus, and cerebellar cortex (Fremeau, Jr. et al., 2004). VGLUT-2, on the other hand, is expressed in the thalamus, brain stem, and deep cerebellar nuclei (Fremeau, Jr. et al., 2004). Synapses with VGLUT-1 expression usually show a lower probability of neurotransmitter release while those with VGLUT-2 expression show a higher probability of release (Fremeau, Jr. et al., 2004; Wojcik et al., 2004). However, none of the brain regions exclusively express only one isoform of VGLUT, and co-expression of both VGLUT-1 and VGLUT-2 can occur (Wojcik et al., 2004). Interestingly, there is a developmental switch from VGLUT-2 to VGLUT-1 expression in the hippocampus, cortex, and cerebellum, with expression levels of VGLUT-2 dominating during the first two weeks of post-natal development (Wojcik et al., 2004). VGLUT-1 knockout mice show normal development during the first two weeks of life, suggesting that expression levels VGLUT-2, and not VGLUT-1, drive glutamatergic transmission during early brain maturation (Wojcik et al., 2004). This developmental switch in expression levels of VGLUTs could explain the significant decrease in protein expression levels of VGLUT-2, but not VGLUT-1, resulting from mPFC synapsin II knockdown at early developmental stages (PD 24) (**Figures 28A-C, 29A-C, 34B**). Even though VGLUT-1 generally predominates within the mPFC later on

in development, our results indicate that the importance VGLUT-2 expression levels in the maintenance of mPFC neurotransmission must not be underestimated. Results from this study indicate that the hypoglutamatergic state of this neurodevelopmental animal and its associated schizophrenia-like behavioural abnormalities is maintained in post-pubertal stages (PD 65-70) of development through specific reductions in VGLUT-2 expression levels within the mPFC (**Figures 29A-C, 34B**). The hypoglutamatergic state of the animal model is maintained at adulthood by decreased VGLUT-2 expression levels in the mPFC despite (1) normal and higher expression levels of VGLUT-1 in the cortex by adulthood, and (2) VGLUT-2 being the minority of the VGLUTs expressed in the cortex at adulthood (Wojcik et al., 2004). A reduction in mRNA expression level of VGLUT-1 has been found in the dorsolateral PFC of patients with schizophrenia (Eastwood et al., 2005). Expression levels of VGLUT-2 in this same brain region, however, was not quantifiable in human patients due to its low expression levels (Eastwood et al., 2005). Regardless, results from this study emphasize a crucial, but yet under-appreciated, role for mPFC VGLUT-2 in the mediation of core schizophrenia circuitry.

In addition to perinatal development, some researchers propose that the period of puberty may serve as another period of neural maturation which can further organize and shape the behavioral and neurochemical pattern of an organism (Sisk et al., 2005; Romeo, 2003). Changes in expression levels and activity of dopamine D_1 and D_2 receptors have been reported in the adolescent brain (Sisk et al., 2005; O'Donnell, 2010). Striatal and prefrontal cortical dopamine receptors are initially over-expressed during early

adolescence and pruned during late adolescence. In contrast, dopamine receptors within the NAc has been found to increase in expression levels around onset of puberty and remain elevated throughout adolescent development and into adulthood (Sisk et al., 2005). This elevation in dopamine receptor numbers is slightly more pronounced in males than females, but neither increase is dependent on pubertal gonadal hormones (Sisk et al., 2005). Further developmental studies in primates indicate changes in prefrontal cortical circuitry during adolescence, such as an ingrowth of dopaminergic fibers (Sisk et al., 2005; Benes, Taylor, & Cunningham, 2000). Moreover, other studies report that the cortical effect of dopamine D_2 receptors on the excitability of interneurons which was not observed pre-adolescence (< PD 36) was now observed post-adolescence (> PD 50) (Tseng & O'Donnell, 2007). Dopamine fibers converge onto both pyramidal cells and GABAergic neurons within the PFC (Benes et al., 2000). Thus, the remodeling of dopamine circuitry can thus have conceivable effects on regulating systems, including the glutamate and GABA neurotransmitter systems, within the CNS. Seeing as how the synthesis of synapsin II is closely associated with dopamine receptor stimulation, it is conceivable that this remodeling in dopaminergic circuitry during puberty may serve to normalize synapsin II expression levels by post-pubertal (PD 65-70) stages (**Figure 34B**) (Chong et al., 2006; Chong et al., 2002). Results support the conclusion of a remodeling of cortical circuitry during primate adolescence and may provide an explanation towards the currently unexplained phenomenon observed in our neurodevelopmental animal model of schizophrenia, namely the transition of the prefrontal cortical hypoglutamatergic state maintained by a synapsin IIa reduction at prepubertal stages (PD

32-35) to a VGLUT-2 reduction at post-pubertal stages (PD 65-70). Moreover, this reorganization in brain patterns during puberty could further explain the transition in therapeutic response to olanzapine drug treatment, from an attenuation of synapsin IIa levels at prepubertal stages (PD 32-35) to an increase in $GAD_{65/67}$ protein expression levels at post-pubertal stages (PD 65-70). Future studies must be performed to further investigate this neurochemical transition during puberty and its role in the development and reduction of symptoms pertinent to schizophrenia.

Of note, acute olanzapine treatment did not attenuate this decrease in VGLUT-2 expression levels in the mPFC at post-pubertal stages. Olanzapine does not possess affinity for receptors involved in glutamatergic transmission nor affect synthesis of VGLUT-2, and subsequently did not influence the protein expression of VGLUT-2 in its therapeutic mechanism of action (**Figure 34B**) (Section 1.5.2.1.).

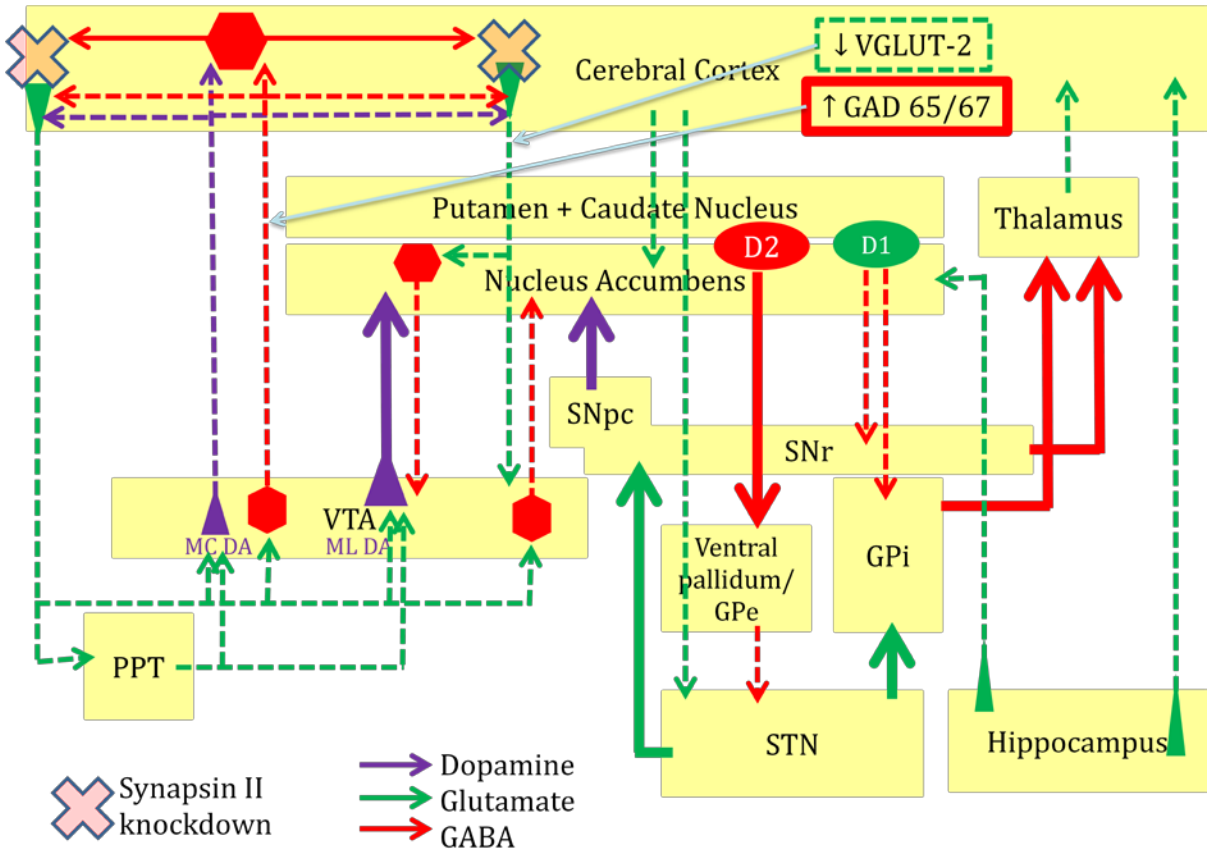


Figure 34B: Proposed maintenance of prefrontal cortical hypoglutamatergic state at post-pubertal stages (PD 65-70). A temporal developmental knockdown of synapsin II (PD 17-23) resulted in lasting reductions in VGLUT-2 protein expression levels in the mPFC at post-pubertal stages (PD 65-70); this decrease in VGLUT-2 protein expression is proposed to maintain the hypoglutamatergic state of the mPFC at this stage of development. Alleviation of hypoglutamatergic state in the mPFC is seen with an olanzapine treatment-induced increase in $GAD_{65/67}$ protein expression at post-pubertal stage (PD 65-70). Light blue arrows indicate the neuron populations in which assessed proteins of interest are hypothesized to act on.

5.3.2. Synapsin II knockdown during PD 17-23 does not induce persistent alterations in the GABA neurotransmission of the medial prefrontal cortex.

VGAT mediates the accumulation of GABA into synaptic vesicles (Chaudhry et al., 1998). Despite predictions of decreased expression levels of VGAT, a majority of studies report VGAT expression levels to be unaffected in schizophrenia. Fung *et al.* suggested a constant ratio of VGLUT-1 and VGAT mRNAs in the dorsolateral PFC of patients with schizophrenia, of which finding parallels the similarly unchanged VGLUT-1/VGAT ratio in our neurodevelopmental synapsin II knockdown model of schizophrenia (Fung, Webster, & Weickert, 2011).

Two isoforms of GAD exists and have been distinguished on the basis of molecular weight, the 65 kDa and 67 kDa isoforms. GAD_{65} has been reported for its specific role in GABA synthesis during postnatal maturation of CNS circuitry, while GAD_{67} is the predominant form present during early development (Stork et al., 2000). GAD_{67} has been preferentially found in the perikarya and dendrites while GAD_{65} has been preferentially found in the axons and terminals (Coyle, 2004; Erlander & Tobin, 1991). GAD_{65} may be responsible for the maintenance of the GABA pool at nerve endings while GAD_{67} contributes to cellular metabolism and the formation of GABA in dendrites and cell bodies (Erlander et al., 1991). However, specified locations for GAD_{65} and GAD_{67} are not mutually exclusive, and GAD_{67} may also be found at the nerve terminals (Erlander et al., 1991). Studies into GABA alterations in the PFC of patients with schizophrenia have thus far been inconclusive. Expression levels of GAD_{65} was found to be normal or only slightly altered in schizophrenia (Lewis, Curley, Glausier, &

Volk, 2012). A number of studies suggest a down regulation in GAD_{67} in the PFC of patients with schizophrenia, of which levels are normalized in medicated patients (Lewis et al., 2012; Veldic et al., 2007; Zink et al., 2004; Akbarian & Huang, 2006). Still other studies suggest differential expression in GAD_{67} depending on the layer of the PFC assessed; layer I showed a decrease in GAD_{67} whereas layer V showed no alterations in GABAergic interneurons (Ruzicka et al., 2007). A reduction of GAD_{67} does not necessarily mean that cortical GABA expression levels are lower in schizophrenia as a down-regulation of GAD_{67} could be the result of reduced GABA metabolism; a case in point is seen when pharmacological inhibition of GABA degradation resulted in elevated cortical GABA and reduced GAD_{67} protein (Lewis et al., 2012). Reductions in GAD_{67} expression levels could also result from a compensatory response to excess GABA activity within the PFC (Volk, Austin, Pierri, Sampson, & Lewis, 2000). The differences could highly depend on the layer of the PFC being examined. Real-time *in vivo* cortical GABA levels are, unfortunately, difficult to measure and much remains to be established surrounding the role of GABA and its associated enzymes in the schizophrenia-afflicted PFC (Lewis et al., 2012). Chronic treatment with dopamine D_2 antagonists have been found to enhance GAD_{67} expression levels, although mechanisms surrounding this therapeutic increase in GAD_{67} expression levels in unclear (Kalkman et al., 2003). Olanzapine treatment could likely cause this therapeutic increase in $GAD_{65/67}$ expression levels via antagonism at the dopamine D_2 receptor in our neurodevelopmental animal model. Enzyme $GAD_{65/67}$ is also closely modulated by glutamatergic transmission via the NMDA receptors, such that a blockade of NMDA receptors and subsequent

hypoglutamatergic transmission may serve to diminish expression GAD_{67} expression (Kalkman et al., 2003). Even though there were no deficits in $GAD_{65/67}$ expression in this current hypoglutamatergic neonatal animal model of schizophrenia, olanzapine treatment was found to induce an increase in $GAD_{65/67}$ expression levels in its mechanism of action, which was found to be sufficient and therapeutic in the normalization of behavioural deficits stemming from hypoglutamatergic function in the neurodevelopmental synapsin II knockdown rats.

5.3.2.1. Olanzapine attenuates schizophrenia-related behavioural abnormalities through alterations in GABA neurotransmission of the medial prefrontal cortex.

A reduction in GAD_{67} expression levels has been found to result from behavioural sensitization in animals and in the PFC of patients with schizophrenia. This reduction in GAD_{67} expression levels have been thought to result in increased excitability of midbrain dopamine neurons through the defective GAD_{67}-induced alterations in cortical glutamatergic projections (**Figure 4B**) (Kalkman et al., 2003). Our newly designed neurodevelopmental synapsin II knockdown model of schizophrenia shows neither significant deficits in $GAD_{65/67}$ nor VGAT protein expression levels in the mPFC. However, acute olanzapine treatment was found to significantly increase protein expression levels of $GAD_{65/67}$ in the mPFC at post-pubertal stages (PD 65-70), in a similar manner to what has been previously reported with chronic treatment of dopamine D_2 antagonists (Kalkman et al., 2003). This emphasizes the therapeutic value of antipsychotic drug-induced enhancement of $GAD_{65/67}$ expression levels in the modulation

of psychosis (**Figure 4B, 34B**). In olanzapine's broad spectrum of receptor targets, pinpointing one specific pathway surrounding its therapeutic modulation of $GAD_{65/67}$ expression in the PFC can be tricky. A few theories surrounding olanzapine's therapeutic mechanism of action can be hypothesized. $GAD_{65/67}$ expression levels have been found by studies to be closely tied to dopamine receptor stimulation. Dopamine D_2 receptor stimulation has been found to cause a decrease in GAD_{67} expression levels while D_1 receptor agonists and D_2 receptor antagonists increased GAD_{67} expression levels (Lindefors, 1993; Laprade & Soghomonian, 1995). Olanzapine's antagonism for the dopamine D_2 receptor may facilitate this increase in $GAD_{65/67}$ expression levels. Since dopamine D_2 stimulation has been found to result in diminished GAD_{67} expression levels in animals, it is intuitive that dopamine D_2 antagonism with olanzapine administration could have an opposite effect of enhancing the production of $GAD_{65/67}$ expression and attenuating the resulting schizophrenia-related abnormalities (**Figure 4B, 34B**) (Kalkman et al., 2003). Olanzapine also binds weakly to GABA-A receptors, and chronic olanzapine treatment has been shown in studies to induce an increase in GABA-A receptors in the PFC (Skilbeck, O'Reilly, Johnston, & Hinton, 2007). This increased modulation of GABA-A receptors can possibly promote an increase in enzyme $GAD_{65/67}$ for increased synthesis of available GABA ligand within the PFC. Finally, the therapeutic mechanisms and low EPS profile associated with use of atypical antipsychotic drugs, like olanzapine, has been attributed not only to its weak antagonism for the dopamine D_2 receptor, but also its potent 5-HT_{2A} receptor antagonism (Meltzer et al., 2003). The serotonin 5-HT_{2A} receptor is widely distributed in the brain with the highest

concentrations located in the cortex (Meltzer et al., 2003). 5-HT_{2A} receptors are located on glutamatergic pyramidal neurons in the cortex, GABAergic interneurons in the cortex and hippocampus, and dopaminergic cell bodies in the ventral tegmentum and substantia nigra (Meltzer et al., 2003). The antagonistic binding of olanzapine for the serotonergic 5-HT_{2A} receptors on GABA interneurons can serve to modulate GABA release and regulate the inhibition of neurons (Meltzer et al., 2003). Antagonism of 5-HT_{2A} receptors located on pyramidal neurons can also serve to alleviate hypoglutamatergic activity within the cortex (Meltzer et al., 2003). A study by Willins *et al.* reported that a majority of the 5-HT_{2A} receptors within the PFC were located on pyramidal neurons while fewer 5-HT_{2A} receptors were found co-localized with interneurons (Willins, Deutch, & Roth, 1997). Moreover, 5-HT_{2A} receptors located in the substantia nigra and VTA can alter the mesocortical, mesolimbic, and nigrostriatal release of dopamine (Meltzer et al., 2003). Interestingly, 5-$HT_{2A/2B/2C}$ antagonism alone only resulted in small increases of cortical dopamine. However, a combination of 5-$HT_{2A/2B/2C}$ and dopamine $D_{2/3}$ receptor antagonism was able to facilitate augmented release of dopamine within the PFC without affecting the striatal dopamine concentration (Meltzer et al., 2003). Olanzapine's antagonism for 5-HT_{2A} receptors located on cortical GABA and glutamate neurons may, through cortico-cortical and cortico-subcortical interactions, provoke the therapeutic increase in $GAD_{65/67}$ and subsequently alleviate glutamate hypofunction within the cortex (**Figure 34B**).

5.3.3. Synapsin II knockdown during PD 17-23 does not induce persistent alterations in the dopamine neurotransmission of the medial prefrontal cortex.

Bogen *et al*. has previously shown that synapsin I and II knockout rodents display decreases in glutamatergic vesicular transporters (VGLUT-1, VGLUT-2) and GABAergic vesicular transporter (VGAT) in the forebrain of the animal (Bogen et al., 2006). This decrease in expression levels of vesicular transporters is comparable to the decreased uptake of the relevant neurotransmitters and reduced transmission in the pathways involved (i.e. glutamate, GABA). On the contrary, dopaminergic VMAT-2 and dopamine uptake was not affected by a double knockout of synapsin I and II. VMAT-2 is responsible for the intracellular transport of monoamines into synaptic vesicles in all monoaminergic neurons (Taylor, Koeppe, Tandon, Zubieta, & Frey, 2000). Previous studies from our laboratory similarly indicated that a specific knockdown of synapsin II did not cause a change in VMAT-2 protein expression levels within the mPFC (Dyck et al., 2011). PET imaging in patients with schizophrenia revealed no significant changes in VMAT-2 binding when compared to control subjects (Taylor et al., 2000). Moreover, VMAT-2 protein has remained insensitive to the regulation of antipsychotic medication (Taylor et al., 2000). It is possible that smaller changes in VMAT-2 binding are undetected in our study, although the absence of a positive finding in changes of prefrontal cortical VMAT-2 expression levels in this study parallels what other researchers have found (Taylor et al., 2000).

DAT is a membrane-spanning protein critical for controlling the availability of dopamine concentrations at the synapse (Lewis, 2000). DAT pumps extracellular dopamine out of the synapse and back into presynaptic terminals for vesicle packaging and subsequent exocytosis (Lewis, 2000; Daniels, Williams, Asherson, McGuffin, & Owen, 1995). In fact, the DAT knockout mice has been shown to display marked excess in dopamine activity and a compensatory downregulation of tyrosine hydroxylase resulting from an absence in DAT (Lewis, 2000). Neuroimaging and post-mortem schizophrenia studies have found reduced dopaminergic innervation and metabolism in the dorsolateral PFC of patients with schizophrenia (Egan et al., 2001). No allelic association of susceptibility was, however, found between schizophrenia and polymorphisms of the DAT gene (Daniels et al., 1995). Importantly, results from this study indicate that that dopamine alterations within the PFC is not a primary mechanism contributing to psychosis and the associated schizophrenia-like abnormalities. In addition, results also indicate that the therapeutic modulation of this same dopamine signalling within the PFC is not fundamental to therapeutically managing symptoms of the disease.

5.4. Limitations

Within this newly developed synapsin II neonatal animal model of schizophrenia lies several unavoidable limitations. Due to the nature of the invasive surgical procedures required to induce a knockdown of synapsin II within the prefrontal cortex during crucial neurodevelopmental periods, it is difficult to establish neonatal control groups which are

unaffected by either the surgical procedures or control solutions infused (i.e. aCSF, sense antisense deoxyoligonucleotide). Secondly, the development of this neonatal synapsin II model of schizophrenia is an extensive study which requires the growth of newly borned neonatal animals into adult stages of development, a process of which takes a minimum of 3 months for each individual study. Lastly, given the large number of animals required for each treatment group at the various pre-pubertal and post-pubertal stages, it is impossible have the required experimental numbers in a single experiment. Rather, a combination of several separate experiments must be performed to attain the necessary numbers for statistical power.

5.5. Future directions

Although this newly developed animal model of schizophrenia has greatly enhanced our knowledge on the causal role of developmental synapsin II expression on the development of schizophrenia-like behaviours in adulthood, as well as the prominent pathways surrounding deficits seen in and therapeutic effects for schizophrenia, much remains to be discovered. First, expression levels of other synapsin genes (I, III) must be determined to assess if there is a compensation of synapsin II function from early in development. Additional behavioural tests assessing the different cognitive domains within this animal model can be performed, as well as neurochemical assessments observing real-time changes in neurotransmission of the various signalling systems. The study of neurochemical changes within additional brain regions proposed to be affected by hypoglutamatergic function within the PFC, such as the striatum, VTA, NAc, and

thalamus, also remains to be established (**Figure 4B**). The dimerization patterns of synapsins in the pathophysiology of schizophrenia would also be an another interesting route to pursue in future studies (Gitler et al., 2004; Monaldi et al., 2010; Hosaka & Südhof, 1999). Although our results indicate the production of schizophrenia-like symptoms to correlate with PFC development, the role of hormonal changes during puberty cannot be ignored entirely. Future studies can be designed to further explore the role of hormonal changes, as well as possible organizational and structural alterations during puberty which may contribute to the remodeling of the cortical system and neurocircuitry pathways in schizophrenia.

In light of recent studies which have demonstrated autism-related abnormalities in synapsin knockout mice, future studies must be performed to elucidate between autism-like and schizophrenia-like behaviours within this newly developed animal model (Greco et al., 2013). Autism and schizophrenia are both neurodevelopmental disorders in a spectrum of mental disorders in which symptom overlap exists. However, minor differences such as a difference in age of onset between the two disorders, or the slightly pronounced presence of hyperactivity in schizophrenia and stereotypy in autism, exists (Tordjman et al., 2007). Additional behavioural tests can be performed within this novel neurodevelopmental synapsin II animal model to assess the predominant features within the present model, as well as to determine if synapsin II alterations within the PFC is specific to schizophrenia, or exists as a gene responsible for symptom overlap between schizophrenia and autism.

6. CONCLUSIONS

1. We provide evidence for a novel model of schizophrenia, in which subtle but selective interruptions in synapsin II gene expression during a critical period of development, is sufficient to cause lasting schizophrenia-like changes in adulthood. We find that the critical period for synapsin II expression is during the stage of maximal expression (PD 17-23) and not the initiation of expression (PD 7). Synapsin II knockdown during the initiation of expression (PD 7) is insufficient to cause lasting schizophrenia-like symptoms into pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages. Only subtle changes in synapsin II gene expression during this critical period of PD 17-23 is sufficient to cause the production of a self-sustaining hypoglutamatergic animal model of schizophrenia, and the resulting production of lasting schizophrenia-like abnormalities into pre-pubertal and post-pubertal stages. The development of schizophrenia-like abnormalities is critically dependent on the timing of synapsin II gene expression and synapse formation during neurodevelopment.

2. Symptoms in this neurodevelopmental model of schizophrenia likely manifest as a function of brain development and is independent of hormonal changes. Schizophrenia-like behavioural abnormalities (i.e. locomotor hyperactivity, social withdrawal, prepulse inhibition deficits) are present both at pre-pubertal (PD 32-35) and post-pubertal (PD 65-70) stages, with equal severity at both stages. This manifestation of symptom classes were thus found to be uninfluenced by hormonal changes during adolescence, but rather revolve around the maturation and synaptic pruning stages of the target brain region of

interest, the mPFC (PD 30 onwards). Furthermore, similar trends in behaviour were found in both male and female populations of animals at both pre-pubertal and post-pubertal stages of development, further disregarding the significant impact of gonadal hormonal surges and fluctuations at puberty on the production of symptoms pertinent to schizophrenia. A remodelling of dopaminergic circuitry in the PFC during puberty could, however, influence the transition between a synapsin IIa-mediated to a VGLUT-2-mediated hypoglutamatergic state within the PFC both at pre- and post- pubertal stages, respectively.

3. This neurodevelopmental synapsin II knockdown model of schizophrenia bears **face validity**. A neurodevelopmental knockdown of synapsin II was found to be causal in the production of schizophrenia-like behaviour in adulthood. These abnormal schizophrenia-like behaviours include hyperlocomotor activity, social withdrawal, and deficits in prepulse inhibition, which are present at both pre-pubertal and post-pubertal stages. These behavioural alterations in this synapsin II neurodevelopmental model of schizophrenia bear resemblance to behavioural findings found in established models of this disease to date, as well as correlate with findings in the human clinical population.

4. Our neurodevelopmental synapsin II knockdown model of schizophrenia presents partial **construct validity**. A physical reduction of synapsin II gene expression is unlikely to occur in the in the developing brain of a human during the third trimester of pregnancy. However, synapsin II reductions have been found in the dorsolateral PFC of patients with

schizophrenia. A more likely event is the genetic predisposition in a patient which would provoke a reduction of synapsin II from birth, and/or suboptimal synapse establishment due to abnormalities in synapsin II expression in affected individuals. Similarly to what is found in animal models of and patients with schizophrenia, hypoglutamatergic function within the mPFC is maintained as the causal mechanism for the development of schizophrenia-like behavioural abnormalities. This hypoglutmatergic function is maintained by a reduction in the protein expression levels of the synapsin IIa isoform during pre-pubertal (PD 32-35) stages, and a reduction in the protein expression levels of VGLUT-2 during post-pubertal (PD 65-70) stages. The maintenance of hypoglutamatergic function in the PFC by differing mechanisms at pre-pubertal and post-pubertal stages suggest a possible influence of synapse re-organization during puberty. Other novel findings from this study include the finding that the synapsin IIa isoform and/or VGLUT-2 is relevant for the maintenance of hypoglutamatergic activity within the PFC, and that a reduction of this protein alone is sufficient to induce lasting schizophrenia-like behavioural changes and sustain neurochemical alterations into pre-pubertal and post-pubertal stages of development.

5. Predictive validity has been further presented in this neurodevelopmental synapsin II knockdown model of schizophrenia. Acute olanzapine treatment was found to effectively normalize all domains of behavioural abnormalities present in this animal model of schizophrenia. Olanzapine treatment prevented hyperlocomotor activity, social withdrawal, and deficits in prepulse inhibition resulting from synapsin II developmental

knockdown. Olanzapine alleviated hypoglutamatergic activity within the mPFC in this neurodevelopmental model of schizophrenia through two differing mechanisms: (1) Increasing glutamate transmission by increasing synapsin IIa protein expression at pre-pubertal stages (**Figure 34A**), and (2) diminishing inhibition on glutamate signalling by increasing $GAD_{65/67}$ protein expression at post-pubertal stages (**Figure 34B**). Future studies can assess the efficacy of other types and classes of antipsychotic drugs, as well as their mechanisms to similarly reduce the presence of these schizophrenia-like abnormalities within this animal model of schizophrenia.

6. This novel neurodevelopmental synapsin II knockdown model differs from the previously founded adult synapsin II knockdown model in our laboratory. The current model also presents several advantages over other established animal models of schizophrenia: (1) Temporal synapsin II knockdown during development is sufficient to cause specific, and lasting changes in behaviour despite the cessation of a knockdown agent by adulthood. On the other hand, the previously developed animal model of a synapsin II knockdown in the adult rat did not have sustained behavioural changes post-cessation of the synapsin II knockdown agent. Moreover, pharmacological models of schizophrenia induce symptoms which are similarly non-permanent and disappear upon drug cessation. (2) The current synapsin II developmental model assesses the combination and interplay of multiple factors contributing to the development of disease, including a genetic predisposition, neurodevelopmental influences, synaptic changes, glutamate hypofunctionality, as well as the therapeutic value of glutamate and/or GABA

signalling. Many different animal models of schizophrenia only mimic parts of the total etiology, and from a standpoint of reductionism, our understanding of the disease can be taken as a sum of the separate constituents of the disease and the various animal models of the disease used. Results from this study provide incredible value to understanding the specific effects developmental expression levels of synapsin II within the PFC have on the production of schizophrenia-like symptoms. This newly developed animal model may also provide a comprehensive platform to design and develop effective therapeutics devoid of side effects.

7. REFERENCE LIST

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