THE EFFECTS OF SYNAPSIN II KNOCK-DOWN IN THE RAT MEDIAL PREFRONTAL
CORTEX ON ATTENTION

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CORTEX ON ATTENTION

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A Thesis

Submitted to the School of Graduate Studies in Partial Fulfillment of the

Requirements for the Degree

Master of Science

McMaster University

MASTER OF SCIENCE (2017)   McMaster University

TITLE: The Effects of Synapsin II Knock-Down in the Rat Medial Prefrontal Cortex on Attention and Vigilance

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NUMBER OF PAGES: xiv, 48

ABSTRACT

It has been estimated that approximately 300,000 Canadians are afflicted with schizophrenia (SCZ). Due to the severity of symptoms as well as critical age of onset, the quality of life among SCZ patients can be poor; thus, further therapeutic research is of great interest.

In addition to the more-common rodent models of SCZ (amphetamine sensitization, PCP sensitization, etc.), our lab has proposed the medial prefrontal cortex (mPFC) synapsin-II knock-down (KD) model. Prior to this study, the cognitive effects of mPFC synapsin-II KD had yet to be reported.

Using a 14-day continuous infusion of antisense (AS) deoxyoligonucleotides for synapsin-II to the mPFC, the results of the KD model have been recreated for further study. The 5-choice-serial-reaction-time-task was utilized to determine the effects of mPFC synapsin-II KD on attention and vigilance. In addition to reductions in both PPI ($p < 0.05$) and social interaction ($p < 0.05$), as well as hyper-locomotion ($p < 0.05$); rats treated with synapsin II AS performed significantly worse on the 5-CSRTT than did control (mismatch/aCSF) animals. The AS animals were significantly less likely to make correct responses ($p < 0.001$), and significantly more likely to commit omissions ($p < 0.0001$) and perseverative responses ($p < 0.05$) than were control animals.

Additionally, an [18F]FDG tracer and PET/CT scans were used to determine differences in brain metabolism due to synapsin-II KD. Results revealed significant reductions in [18F]FDG among AS treated rats ($p < 0.05$) when compared to control animals. This reduction appeared to be a global result, however it followed 13-days of treatment which may account for the widespread effect.

The mPFC synapsin-II KD model of SCZ has showcased cognitive and metabolic effects similar to that of SCZ. These findings, in conjunction with past research, provide evidence for the synapsin-II KD model as a viable rodent model of SCZ. Further research utilizing this model will provide valuable insight into the pathogenesis of SCZ and potential therapeutics.

ACKNOWLEDGEMENTS

First and foremost I would like to thank Dr. Mishra. You have been an outstanding PI. You have shown more patience and understanding than I could have ever asked for. Thank you.
I would also like to thank my supervisory committee members, Dr. Szetchman and Dr. Selvaganapathy, for all of their help and guidance over the years.
Third, I need to thank everyone I worked alongside in the lab during my time here. Ritesh, Asim, Kristen, Yogesh, Pat, Tiffany, Jay, Sharron, Aaron, Mattea, Sharnpreet, Dipa, Christal, Farhat, Heta, Ashley, Roohie, Shreya, Joella, Edwin, Omar, Raph and Aisha. Thank you so much for all your help and the ability to put-up with me for 5 years!
I also owe the staff of the CAF a great thanks as you have worked as hard for this thesis as anyone. Specifically Tammy, myself (and the rats) cannot express how thankful I am for all your hard work and care that it takes to look after these fur-balls.
I would also like to thank Sandra Murphy for her persistence and patience. I'm sorry some paperwork took forever for me to finish.
Finally, Crystal, thank you more than you know. Without you, I do not know if this would have been possible. You were more instrumental in this than I can convey here. I will never forget what you have sacrificed to help me.

Table of Contents

List of Tables

List of Figures

LIST OF ABBREVIATIONS
5-HT…………………..5-hydroxy tryptamine (Serotonin)
8-ARM…………… eight-arm radial maze
aCSF……………… artificial cerebral spinal fluid
ADON…………...antisense deoxyoligonucleotides
ANOVA …………analysis of variance
A/P ………………..anterior-posterior
AP-2α……………...Activating protein 2-alpha
APD………………anti-psychotic drug
AS…………………..antisense oligonucleotides
ASD………………..Autism Spectrum Disorder
BD…………………. Bipolar disorder
CAF………………… Central Animal Facility
CamK…………… calmodulin-dependent protein kinase
CAMP…………………cyclic adenosine monophosphate
CBT…………………….cognitive behavioral therapy
CT………………….. computerized tomography
D1R……………….. dopamine D1 receptor
D2R……………….. dopamine D2 receptor
DA………………… dopamine
DISC1…………….. disrupted-in-schizophrenia 1
DLPFC……………. dorsolateral prefrontal cortex
ECL………………… enhanced chemiluminescence
EGR-1…………... Early growth response factor-1
18F-DOPA……… 18F-dihydroxyphenyl-L-alanine
[18F]FDG………. fluorine-18-fluorodeoxyglucose
GABA……………… γ-aminobutyric acid
GAD……………….. glutamic acid decarboxylase
GAPDH…………… glyceraldehyde 3-phosphate dehydrogenase
GPe………………… globus pallidus external

GPi…………………. globus pallidus internal
IDT…………………. Integrated DNA Technology
M/L………………… medial-lateral
mPFC……………… medial prefrontal cortex
NIMH……………… National Institute of Mental Health
NMDA……………. N-methyl-D-aspartate
NMDAR………….. N-methyl-D-aspartate receptor
nVH………………… neonate ventral hippocampus
OD………………….. optical density
PEA-3………………polyoma enhancer activator 3
PKA………………… protein kinase A
PBS…………………. phosphate buffered saline
PSB-T………………. phosphate buffered saline with Tween®20
PCP…………………. phencyclidine
PD…………………… post-natal day
PET………………….. positron emission tomography
PFC………………….. prefrontal cortex
PPI…………………… prepulse inhibition
PS……………………. phosphorothioate
PVDF……………….. polyvinylidene fluoride
SDS-PAGE……………. sodium dodecyl sulfate-polyacrylamide
SN……………………….. substantia nigra
SPECT………………….. single photon emission computed tomography
STR………………………. striatum
STN………………………. subthalamic nucleus
SynII……………………..Synapsin II
TBS………………………. tris buffered saline
TBS-T…………………… tris buffered saline with Tween®20
VH……………………….. ventral hippocampus

DECLARATION OF ACADEMIC ACHIEVEMENT
All work on this project was completed by myself, Luke Molinaro, unless otherwise specified. Biochemical analysis completed by Tiffany Tian and Patricia Hui. 5-CSRTT data collection and training was completed with the assistance of Sohel. Maze data collected by Sharnpreet Krooner and Sharon Thompson. Imaging data analysis was completed with the assistance of Ashley Bernardo. Imaging protocol created by Ritesh Daya, Heta Joshi, and the imaging facility at McMaster.

SECTION 1: INTRODUCTION

1.1 Schizophrenia

Schizophrenia (SCZ) is a psychiatric illness that afflicts upwards of ~1% of the Canadian population (Canadian mental health association, 2016). At that rate, it is estimated that 300,000 Canadians are affected by this disease. The total combined cost, both healthcare and non-healthcare related, have been estimated at $6.87 billion CAD every year. In addition to the monetary cost of dealing with SCZ, patients diagnosed with SCZ often experience social (including occupational) and emotional dysfunction. Patient populations consist equally of men and women, with slight variation in average age of onset and diagnosis. Men will often present with SCZ in their late teens to early 20's, while women tend to present slightly later in their 20's and early 30's (Schultz et al., 2007). Due to the previously mentioned hardships, in addition to the critical age of onset, prognosis with SCZ is often poor and results in reduced quality of life.

1.1.2 Symptoms

The symptoms of SCZ often differ on an individual scale but have generally been separated into three broad categories; positive symptoms, negative symptoms, and cognitive symptoms. Put simply; the positive symptoms encompass aspects gained as a result of the disease. This includes hallucinations, delusions, and paranoia among others (Schultz et al., 2007). Contrarily, the negative symptoms represent aspects lost or reduced due to the disease. Negative symptoms include (but not limited to) anhedonia (lack of pleasure), social withdrawal, and reduced or blunted affect (Kirkpatrick et al., 2006). Cognitive symptoms, as the name implies, include those which result in reduced cognitive abilities including; working memory deficits, attentional deficits and an inability to problem-solve or reason (Bozikas et al., 2006).

1.1.3 Treatment

Despite advances in the treatment of SCZ over the past 20 years, no cure for the disease exists. The most efficacious treatment to date is a combination of anti-psychotic drug (APD) therapy and cognitive behavioral therapy (CBT) which often includes social skill and reasoning therapies (Bozikas et al., 2006). There are two general classes of APDs used in the treatment of SCZ; first generation or "typical" APDs (ex. Haloperidol) and second-generation or "atypical" APDs (ex. Olanzapine). These two classes of APDs also differ in their pharmacological mechanism of action, treating SCZ in differential manners. Typical APDs primarily bind to dopamine (DA) D2 receptors, while atypical APDs act on DA D2 and 5-HT (Serotonin) receptors (Bozikas et al., 2006).

1.1.4 Etiology

To date, the exact pathogenesis of the disorder has not been elucidated. There are, however, a number of hypotheses that have been put forward. Evidence in the literature suggests that the neurotransmitters dopamine, glutamate, and GABA, are involved in, and play an integral role in the complex etiology of SCZ (Cohen et al., 2015).

The dopamine hypothesis postulates that hyper-dopaminergic activity in the striatum is connected with the positive symptoms of SCZ, and hypo-dopaminergic activity in the cortical regions with the negative and cognitive symptoms. Evidence for this has been provided by use of pharmacological agents, such dopamine agonists (e.g., amphetamine), which induce SCZ-like symptoms in humans. In vivo positron emission tomography (PET) imaging studies utilizing 18F-dihydroxyphenyl-L-alanine (18F-DOPA) tracers have demonstrated elevated uptake levels in the substantia nigra and striatum of schizophrenic patients. These results suggest an increase in DA synthesis, in nigral and striatal regions, in SCZ (Howes et al., 2013). These dopaminergic pharmacological agents are also common inducers of SCZ-like behaviour in preclinical animal models. In addition, single photon emission computed tomography (SPECT) and PET studies have shown that patients with SCZ show elevated synthesis and release of DA in the basal ganglia (Thierry et al., 2000). Additionally, under-stimulation of the DA-D1 receptors and low DA activity in the PFC has been suggested to correlate with cognitive impairment and poor performance in tasks involving working memory seen in patients with SCZ (Thierry et al., 2000; Laruelle et al., 2003; Tamminga and Holcomb, 2005). Therefore, regulation of synaptic proteins by the dopamine receptor is not unexpected.

The glutamate hypothesis of SCZ was postulated due to the effects of N-methyl-D-aspartate (NMDA) receptor antagonists such as ketamine and phencyclidine (PCP). Chronic use of these drugs can result in manifestations of the symptoms that characterize SCZ. Administration of NMDA agonists such as glycine and D-serine has been shown to reduce the severity of symptoms in patients with SCZ (Greengard et al., 1993; Laurelle, 2003). Due to the above mentioned findings, it was hypothesized that NMDA hypofunctionality is implicated in the pathogenesis of SCZ (Goff & Coyle 2001; Tammings & Holcomb, 2005).

The hyperdopaminergic and hypoglutamatergic activity suspected to be responsible for SCZ may work in a complementary fashion. Prolonged NMDA hypofunction has been found to cause a reduction in mesocortical DA transmission and subsequent increase in subcortical mesolimbic DA activity, trends that are consistent with the DA hypothesis of SCZ (Laurelle et al., 2003). The synaptic dysfunction within the PFC may lead to sustained dysregulation of glutamate signalling and the ensuing secondary signalling abnormalities (ex. subcortical dopamine hyperactivity and cortical DA hypoactivity) (Laurelle et al., 2003) [**Figure 1.0**].
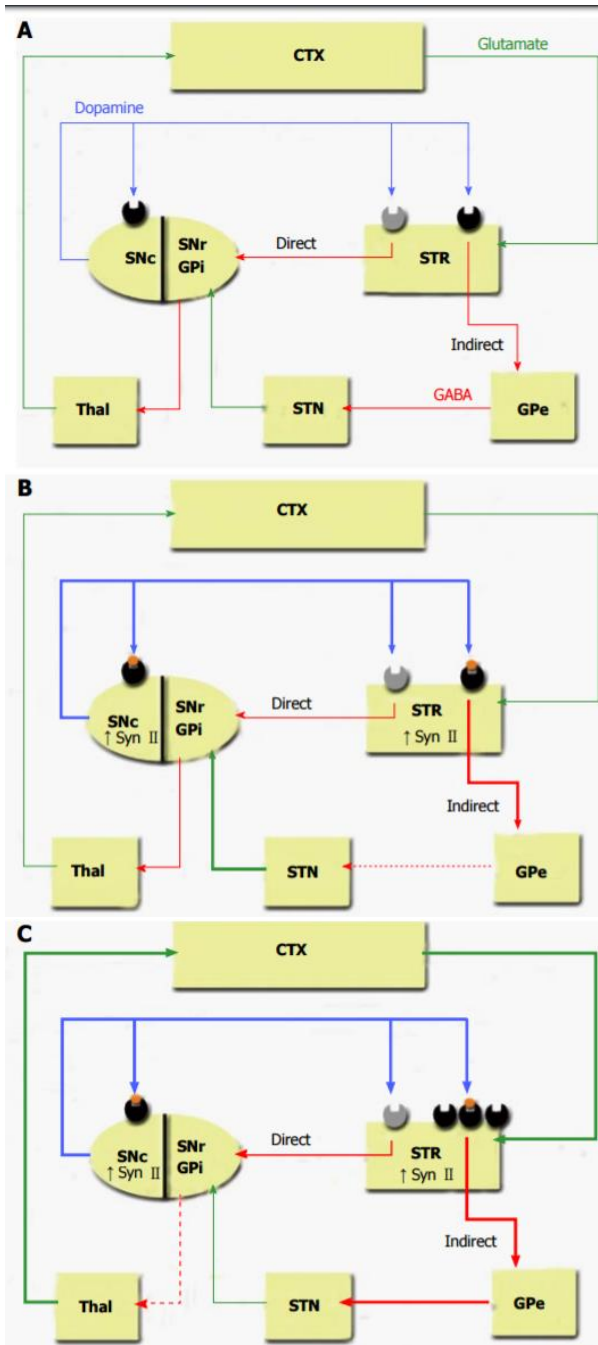
Figure 1: Depiction of basal ganglia interconnectivity. Diagrams showcase both the direct and indirect pathways. A: Normal basal ganglia regulation; B: Basal ganglia regulation following subchronic haloperidol treatment. Synapsin II levels also indicated; C: Basal ganglia regulation following chronic haloperidol treatment and manifestation of EPS. Synapsin levels are also indicated. Difference in neurotransmitter output is indicated through the various line colours (green = glutamatergic, red = GABAergic, blue = dopaminergic). Line thickness is reflective of neurotransmitter activity (thicker lines represent increased activity, dotted lines represent reduced activity). The various impacted brain regions are represented within boxes. Ghose and Tamminga. Handbook of Contemporary

Neuropharmacology 2007: 251-283. CTX: Cortex; GPe: Globus pallidus external segment; GPi: Globus pallidus internal segment; SNc: Substantia nigra pars compacta; SNr: Substantia nigra pars reticulata; STN: Subthalamic nucleus; STR: Striatum; EPS: Extrapyramidal symptoms.

1.2 Synapsins

1.2.1 Synapsin family of phosphoproteins

Synapsins were one of the first families of synaptic vesicle-associated proteins identified and characterized. The Synapsins are the most highly abundant family of neuron-specific phosphoproteins that have been well conserved evolutionarily. Synapsins make-up 9% of all vesicular proteins (Sudhoff et al., 1989; Sudhoff et al., 2004; Greengard et al, 1993). Additionally, Synapsins function presynaptically through a variety of roles, including synaptogenesis, synaptic plasticity, synapse function, and synaptic maintenance.

One role of particular interest played by the synapsin phosphoproteins is the regulation of synaptic vesicles (Hilfiker et al., 1999). Synapsins interact with actin filaments to actively, and selectively, bind synaptic vesicles to the cytoskeleton at the site of the reserve pool (Greengard et al., 1993) [**Figure 2.0**]. The influx of Ca2+ ions associated with an action potential cause the phosphorylation of synapsin proteins and the subsequent liberation of vesicles from the cytoskeleton. The vesicles release from the cytoskeleton allows it to migrate from the reserve pool toward the synaptic membrane for exocytosis and subsequent neurotransmitter release (Greengard et al., 1993; Chen et al., 2004). 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).

Synapsins are coded by the SYNI, SYNII, and SYNIII genes; which are located on chromosome X, 3 and 22, respectively (Hilfiker et al., 1999). There are a total of 10 isoforms resulting from variable (alternative) splicing of the aforementioned genes [**Figure 3.0**] (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 neuron type (Bogen et al., 2006; Greengard et al., 1993). Synapsin proteins I and II (syn1 and syn2) are the most common being found in mature synapses, while synapsin III (syn3) is less common but plays a larger role developmentally (Südhof, 2004; Greengard et al., 1993; Hilfiker et al., 1999).
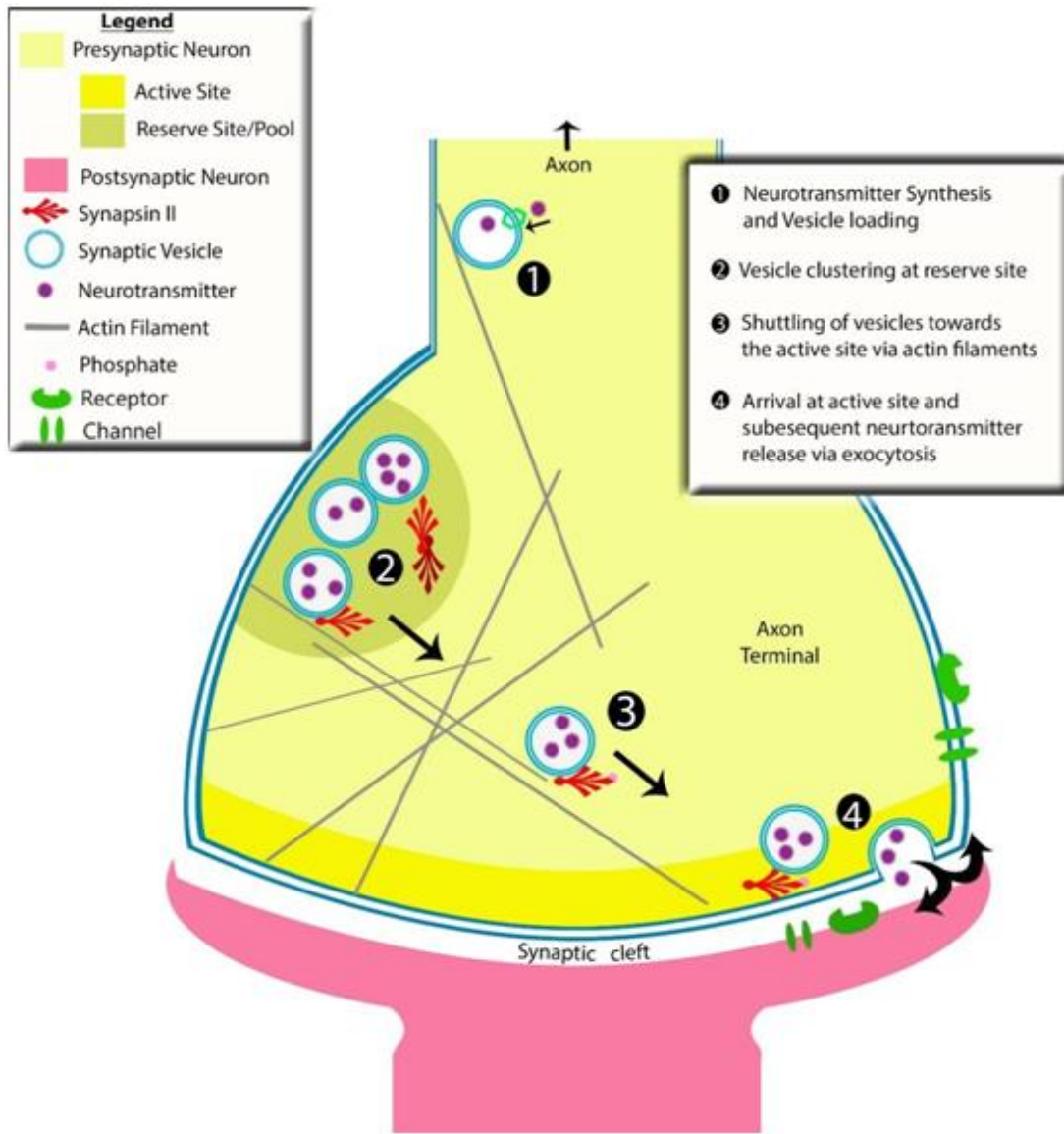
Figure 2.0 A schematic representation of the various functions of Synapsin II in the presynaptic neuron. Included are various structural and protein components as well as the various stages of synapsin II involvement.

Structurally, synapsins are highly conserved throughout most domains. The variable splicing of the various isoforms leads to structural changes located within the COOH-terminus of the protein (Hilfiker et al., 1999; Südhof, 2004). Domains A-C are common to all isoforms of synapsins. Domain E is common to G-isoforms and is thought to cluster synaptic vesicles in/maintain the reserve pool (Südhof, 2004; Fornasiero et al., 2010; Gitler et al., 2008; Greengard et al., 1993; Hilfiker et al., 1998). Domains B, D, and F-J are poorly conserved and specific to each synapsin isoform [**Figure 3**] (Südhof, 2004; Pieribone et al., 1995; Kao et al., 1999; Greengard et al., 1993; Hilfiker et al., 1998). The conserved, 20 residue N-terminus binds to the lipid surface of vesicles. The aforementioned C-terminus, an elongated structure comprised of basic/hydrophilic AAs, binds to the actin cytoskeletal component of vesicles (Sudhof, 2004; Pieribone et al., 1995; Kao et al., 1999; Greengard et al., 1993; Hilfiker et al., 1998). The binding of synapsins is diminished via phosphorylation of a select serine residue in domain A via a range of kinases (Südhof, 2004; Pieribone et al., 1995; Kao et al., 1999; Greengard et al., 1993; Hilfiker et al., 1998). With respect to the previous statement, domain A contains binding sites for cyclic adenosine 3',5'-monophosphate (cAMP)-dependent protein kinase A (PKA), Ca2+/calmodulin-dependent protein kinase I (CaMKI), and CaMKIV (Fornasiero et al., 2010). The C-domain is the largest of the synapsin domains, often accounting for half of the entire protein length, and contains a phosphorylation site for tyrosine kinase Src in synapsin I and II (Südhof, 2004; Fornasiero et al., 2010). The C-domain is also responsible for dimerization and the high-affinity binding of ATP (Südhof, 2004; Fornasiero et al., 2010; Pieribone et al., 1995; Kao et al., 1999; Greengard et al., 1993; Hilfiker et al., 1998). The various types of synapsins behave differently with respect to ATP binding. Synapsin I only binds ATP in the presence of CA2+, synapsin II only binds ATP in the absence of ATP, while synapsin II binds regardless of Ca2+ levels (Südhof, 2004; Hosaka & Südhof, 1998). The D-domain contains phosphorylation sites for CAMK-II, mitogen-activated protein kinase (MAPK) and cyclin-dependant kinase 1/5 (CDK 1/5) (Südhof, 2004; Fornasiero et al., 2010; Schweizer et al., 2006; Skoblenick et al., 2010). Figure 3.0 contains a schematic representation of the various synapsins and their domains.
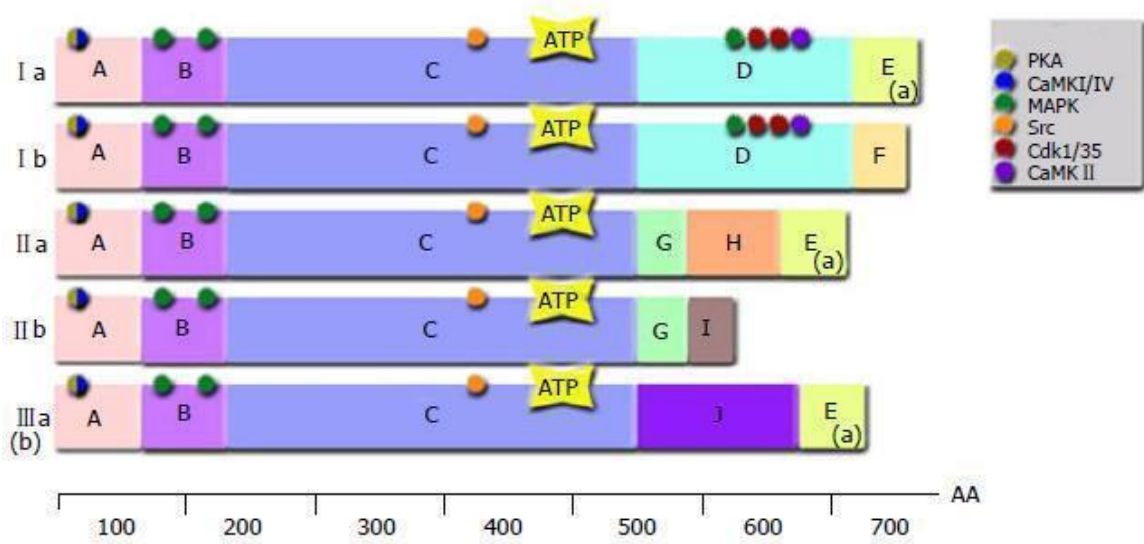
Figure 3.0: Schematic representation of the domain structure of Synapsins.
Included are various transcription factor binding sites.
** Only one isoform for synapsin III is indicated in Figure 3.0, but multiple synapsin III products
have been found in the adult brain (Fornasiero et al., 2010).
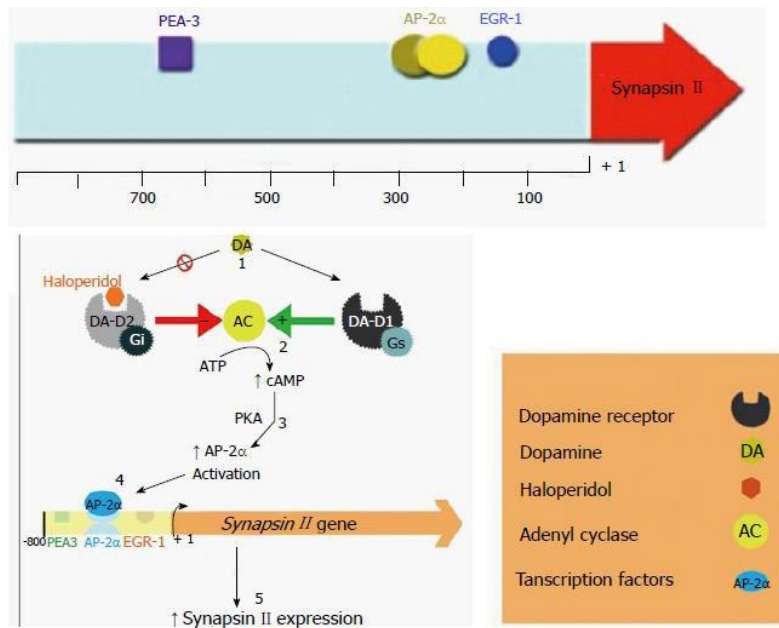
Figure 4.0

Illustration of the promoter regions of synapsin I and II respectively and proposed mechanism of dopaminergic regulation of synapsin II. A: Illustration of the promoter regions of synapsin I and II respectively. Transcription factor binding sites have been indicated, showing their various positions in the promoter region; B: Proposed mechanism of dopaminergic regulation of synapsin II. Evidence: (1) Immunocytochemistry results indicate that ligand - DA Rc binding results in changes to synapsin protein levels dependant on Rc subtype; (2) Ligand binding causes changes to intracellular cAMP levels; (3) PKA inhibitors (5-24 amide trifluoroacetate salt, Rp-cAMPS) cause changes in synapsin II translation; (4) DA-D1 stimulation may cause AP-2 to bind to synapsin II promoter. Synapsin II expression levels were inhibited when cells were treated with AP-2 ADONs. Subsequent treatment with DA-D1 or -D2 agonists showed to effect on synapsin II expression; and (5) Synapsin 2 expression can be altered via upstream alteration at various points. Additional information: (1) EGR-1 levels are not affected by chronic treatment with DA-D1 or DA-D2 antagonists; (2) Antisense deoxyoligonucleotides for AP-2 reduces synapsin II expression levels; and (3) Antisense deoxyoligonucleotides for EGR-1 and PEA3 have no effect on the expression of synapsin II. EGR-1: Early growth response factor-1; PKA: Protein kinase A; cAMP: Cyclic AMP; AP-2α: Activating protein 2-alpha.

1.2.2 Synapsin II

The 5'-flanking region of the synapsin II gene has been found to contain binding sites for transcription factors such as inducible zinc-finger transcription factor, early growth response factor (EGR-1), polyoma enhancer activator 3 (PEA-3), activating protein 2-alpha (AP-2α), and numerous Sp1 sites (Petersohn, Schoch, Brinkmann, & Thiel, 1995). Studies suggest that methylation may have an effect on synapsin II expression as the transcriptional start sites and proximal promoter regions of the synapsin II gene were found to contain GC-rich sequences (Petersohn et al., 1995). [**Figure 4.0**] details the regulation of synapsin II.

Research suggests that synapsin IIa plays a significant role in the modulation of synaptic vesicle pools and signalling in a variety of neurons, specifically glutamatergic neurons (Gitler et al., 2008). Fig. 2.0 showcases the function of synapsin II. Complete synapsin knockout (triple knockout) cells have shown aberrant catecholamine release, which can be ameliorated with synapsin II treatment (Villanueva, Thornley, Augustine, & Wightman, 2006). It has been suggested that synapsin IIa may not be required for catecholamine released, but rather acts as a negative modulator to reduce catecholamine release (Villanueva et al., 2006).

For further information regarding Synapsin II, please refer to:
Molinaro L, Hui P, Tan M, Mishra RK. Role of presynaptic phosphoprotein synapsin II in schizophrenia. World journal of psychiatry. 2015 Sep 22;5(3):260.

1.2.3 Synapsin II and SCZ

The synapsin family of proteins have also been implicated in the pathogenesis of SCZ (Vawter et al., 2002). The synapsin II gene is located on a chromosomal region of vulnerability with respect to SCZ (3p25) (Saviouk et al., 2007; Pulver et al., 1995). Distinct population subtypes have also been found to contain genetic polymorphisms (single-nucleotide polymorphisms and insertion/deletion polymorphisms) positively associated with the development of SCZ (Chen et al., 2004 x2; Saviouk et al., 2005; Lee HJ, et al. 2005). Synapsin II has also been shown to co-localize with CAPON, which has been identified as a major candidate of susceptibility gene for SCZ (Dyck et al., 2011; Zheng et al., 2005). CAPON functions as an adapter protein for nitric oxide synthase (Dyck et al., 2011; Zheng et al., 2005). Due to this, a reduction of synapsin II may result in reduced levels of nitric oxide. Because of the importance of nitric oxide in synaptic transmission, a reduction in synapsin II could possibly contribute to the etiology of SCZ.

Synapsin II dysfunction has further been implicated in the etiology of SCZ with the use of preclinical animal models. Subjects in which a/multiple synapsin(s) are knocked-down often result in a phenotype containing symptoms indicative of established animal-SCZ models. It has been shown that synapsin II knockout mice display behavioral abnormalities in locomotion (hyperactivity), social dysfunction (withdrawal), deficits in sensorimotor gating (PPI) (Dyck et al., 2007; 2008; 2011). Similar studies utilizing rats as subjects have found that reductions of synapsin in the medial prefrontal cortex (mPFC) caused similar behaviors to manifest, in addition to cognitive impairments (in 5-CSRTT and radial 8-arm maze. These results are not mimicked in other areas of the brain, suggesting a unique role of synapsin II in the mPFC (Dyck BA, et al. 2012). In addition to this behavioral work, biochemical work shows a reduction in various glutamate and GABA vesicular transport proteins (VGLUT1, VGLUT2, VGAT) (Dyck 2011).

Finally, the abnormalities produced as a result of synapsin II KD can be ameliorated with the administration of anti-psychotic drugs (APDs) such as olanzapine (Chong et al., 2006; Dyck 2011).

For further information regarding Synapsin II and SCZ, please refer to:
Molinaro L, Hui P, Tan M, Mishra RK. Role of presynaptic phosphoprotein synapsin II in schizophrenia. World journal of psychiatry. 2015 Sep 22;5(3):260.

1.2.4 Involvement of Synapsin II in other diseases

Synapsin II has also been implicated in a number of other mental illnesses, such as bipolar disorder (BD) and autism spectrum disorder (ASD). Genetic variation studies suggest that synapsin II gene mutations (missense or nonsense) may be responsible for the development of ASD (Corradi et al., 2014). Previous studies have found that expression levels of synapsin II mRNA were increased in post-mortem PFC samples of BD patients (Lopez et al., 2010). Additional studies have shown support for a genetic linkage between synapsin II and lithium-responsive bipolar disorder (Chong et al., 2006; Lopez et al., 2010). In vivo studies measuring effects of lithium, one of the most common treatments for BD, on synapsin II levels have proven inconclusive. It has been indicated that some patients show an increase in synapsin II expression following lithium treatment, while other show a decrease in synapsin II expression (Cruceanu et al., 2012). It has been suggested that this may be due to the mood stabilizing effects of lithium, and its functions to normalize variation across different behavioral states (Cruceanu et al., 2012).

In vitro administration of lithium significantly increased expression levels of the synapsin IIa isoform compared to controls in neuronal cell types. Conversely, lithium treatment did not result in any significant changes to synapsin IIb expression (Alda et al., 2009; Cruceanu et al., 2012). Moreover, synapsin II knockout in mice resulted in an epileptic phenotype, stronger at 2-3 mo of age, a period when synapsin II levels are highly expressed and synapses undergo intense maturation and refinement (Greco et al., 2013). Similarly, of other synapsin knockout models, mice lacking synapsin II demonstrated the most robust phenotype of autism, characterized by reduced social interaction, and decreased interest for environmental stimuli (Greco et al., 2013).

For further information regarding Synapsin II and disease, please refer to:
Molinaro L, Hui P, Tan M, Mishra RK. Role of presynaptic phosphoprotein synapsin II in schizophrenia. World journal of psychiatry. 2015 Sep 22;5(3):260.

1.3 Animal Models of SCZ
1.3.1 Overview
As is often the case with preclinical work, animal models are an invaluable tool in study of disease states and possible therapies. The use of such models has been essential in the understanding of SCZ. Not only do these models advance our understanding of the disease state of SCZ, but also possible therapeutics to treat this state (Lipska, 2004; Tseng et al., 2009).

Pharmacological Models: Pharmacological rodent models of SCZ may be the most typical in preclinical research due to both their ease and ability to accurately mimic certain symptoms. Of these models, chronic amphetamine sensitization and chronic phencyclidine (PCP) sensitization, are the most common. Amphetamine is a psychostimulant which has been used throughout history

to treat many conditions (Fleckenstein et al., 2007). Amphetamine has been shown to cause the release of monoamines, specifically DA, by targeting DAT and VMAT-2 among others. The exact mechanism by which amphetamine affects DA levels is unknown, however, it is generally accepted that amphetamines can cause an increase in dopaminergic signalling by preventing its reuptake (Fleckenstein et al., 2007; Calipari et al., 2013). The regime for amphetamine sensitization involves chronic amphetamine administration in intermittent escalating doses, followed by a period of withdrawal (Peleg-Raibstein et al., 2008). Chronic amphetamine administration in rodents was found to induce this sensitization. Once sensitized, subjects display symptoms indicative of SCZ including: hyperactivity induced by acute amphetamine challenge, deficits in prepulse inhibition, and deficits in cognitive-based tasks such as the 5-CSRTT or attentional set shifting task (Peleg-Raibstein et al., 2008). Animals that have been sensitized in this manner have shown increased dopaminergic signalling in the NAc and dorsal striatum, while showcasing deceased signalling among cortical neurons (Featherstone et al., 2007; Peleg-Raibstein et al., 2008). This model provides further validity as it has been found that APDs which act primarily on dopamine receptors are able to ameliorate the previously described symptoms. Furthermore, pre-treatment with either haloperidol or clozapine (APDs) were found to prevent the effects of chronic amphetamine administration (Peleg-Raibstein et al., 2008). While this model replicates many of the positive, and some of the cognitive, symptoms; replicating others (negative and other cognitive symptoms), remains a challenge for this model (Peleg-Raibstein et al., 2008; Mouri, Noda, Enomoto, & Nabeshima, 2007).

Similarly, chronic administration of NMDA non-competitive antagonists (ex. PCP, ketamine, etc.) has also been found to induce a SCZ-like state. It was found that these drugs would induce hallucinations and delusions similar to the psychosis of SCZ, in healthy controls (Jones et al., 2011; Mouri et al., 2007). In addition to this, subjects also presented with progressive social withdrawal, dysfunction of speech/communication, as well as cognitive dysfunction; thus containing all 3 symptom classes of SCZ. (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 strength of the PCP sensitization model is that the symptoms are more-likely to persist beyond the cessation of drug administration. (Jones et al., 2011; Mouri et al., 2007). Chronic PCP sensitization results in an increase in locomotor activity (upon PCP challenge) and a reduction in social interaction, both of which are reversed by the administration of antipsychotic drugs (Jones et al., 2011; Mouri et al., 2007). While it was found that the cognitive effects could be ameliorated by both atypical and typical (atypical is more successful) APDs, the social deficits induced could only be attenuated by atypical APDS while the PPI deficits – inversely – only responded to typical APDS (Jones et al., 2011; Mouri et al., 2007). Biochemically speaking, it has been determined that chronic PCP administration results in a mesolimbic dopamine system that is hyper-responsive to stress and drug challenge, accompanied by a reduction in PFC synapsin II levels (Jones et al., 2011). A final facet worth mentioning with respect to the rodent PCP sensitization model of SCZ, is the resulting cortical glutamate hypofunction and hypometabolism, which can be further characterized as reduced glutamate concentrations (Jones et al., 2011; Mouri et al., 2007). This is of interest as there is growing evidence for glutamatergic dysfunction within the underlying etiology of SCZ (Jones et al., 2011)

The above discussed models only account for two possible pharmacological-rodent models of SCZ, many more exist (ex. chronic MK801 treatment) but in the interest of this thesis

they will not be mentioned.

Genetic models: As discussed previously, there have been multiple at-risk genes identified with respect to clinical populations of SCZ patients (Jones et al., 2011). Of the at-risk genes, most have been implicated in synaptic function, maintenance, plasticity and development; yet no individual gene has been found to sufficiently explain the pathogenesis of SCZ (Jones et al., 2011). With this in mind, it is likely that multiple genes act in a synergistic manner, perhaps during developmental periods that determine the onset and severity of SCZ (Jones et al., 2011). With this in mind, multiple genetic rodent models of SCZ have been developed to further determine their effects in an individual, and synergistic, manner.

Disrupted-in-Schizophrenia 1 (DISC-1) is one of the primary genes to be implicated in the etiology of SCZ. DISC-1 expression is known to play a role in synaptogenesis, synaptic plasticity, and neuronal migration; from very early in development (Jones et al., 2011; Pletnikov et al., 2008). DISC-1 mutants showcase many of the behavioral abnormalities associated with rodent models of SCZ including: Hyperactivity, reduced social interaction, and deficits in working memory and executive function (Hikida et al., 2007; Pletnikov et al., 2008). DISC-1 mutants also present deficits in PPI which have been shown to be ameliorated with APD treatment (Jones et al., 2011). However, this model is not without its disadvantages. For example, not all animals are affected equally by the DISC-1 mutation, nor do they respond equally to treatment. It is thought that this is due to the method of inserting/inducing the mutated gene, as opposed to effects of the gene product itself (Jones et al., 2011).

Another known genetic rodent model of SCZ is the Reelin model. Reelin is a gene integral in synaptic formation and plasticity, which has been shown to be reduced in certain brain regions of SCZ patients (Jones et al., 2011; Fatemi, 2001). Reelin knockout mice showcase a phenotype consisting of marginal cognitive deficits, minor PPI deficits, and a reduction of dendritic spine density in the frontal cortex (Jones et al., 2011; Fatemi, 2001). A drawback to this model is the severity of abnormalities in the mutant rodents, which present in a manner more extreme than what is often seen in SCZ (Jones et al., 2011; Fatemi, 2001).

Another gene showing promise with respect to SCZ development is dystobrevin-binding protein 1 (DTNB1), which has been shown to have a strong correlation between mutations and SCZ manifestations (Jones et al., 2011; Fatemi, 2001). DTNB1 encodes for the protein Dysbindin which has been shown to regulate synaptic transmission via exocytosis, vesicle formation, and receptor trafficking (Numakawa et al., 2004). Dysbindin mutant rodents present a phenotype consisting of hyperactivity, reduced social interactions, and altered working (Jones et al., 2011; Feng et al., 2008). Other symptoms, such as PPI, show inconsistent results in Dysbindin mutants (Feng et al., 2008).

The three aforementioned models of SCZ are not the only rodent genetic models, but rather 3 examples of such. Due to their limited involvement in this thesis, no others will be discussed.

In addition to two types of rodent models previously discussed, there are also developmental based models. These models often include early lesioning, various prenatal exposures/insults (virus, hypoxia, etc. (McGrath et al., 2003). Because this research deals entirely with adult animal subjects, these models will not be expanded upon in this thesis.

1.4 Genetic Knockdown techniques

1.4.1 Overview

Gene manipulation is often utilized 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 are commonly used to manipulate gene expression and determine gene function.

1.4.2 Designed Antisense-oligonucleotide knockdown

In this experiment AS technology is utilized as it provides a method that is easy to execute, low in cost, highly specific and highly consistent in results (Kurreck, 2003). Use of AS as compared to gene knockout technology also offers a higher survival rate of subject animals (Kurreck, 2003). AS are agents utilized to modify the expression of highly specific target gene(s) in a sequence-specific manner (Kurreck, 2003). Oligonucleotide molecules usually consist of 15-20 nucleotides complementary to the target mRNA. AS sequences function via the inhibition of mRNA targets prior to translation (Kurreck, 2003). Pubmed BLAST is an online tool that can be used to determine the specificity of the AS sequence to the target mRNA when creating deoxyoligonucleotide sequences. Sense or mismatch deoxyoligonucleotide sequences are commonly used as options for negative controls (Kurreck, 2003). The 2 mechanisms by which AS sequences inhibit gene expression are:

1. AS can activate RNase H, an enzyme which cleaves the RNA moiety of the DNA-RNA heteroduplex though RNase H-mediated degradation of RNA.
2. The AS sequence can also inhibit translation through steric blockade of the ribosome (Kurreck, 2003; Scherer & Rossi, 2003).

In order to effectively reduce 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 deoxyoligonucleotides are rapidly degraded by nucleases in vitro and in vivo. With this in mind, strategies have been developed to mitigate this degradation. Modifications include the addition of specialized sugars at the 2' ribose position, or altered phosphate backbones such as added phosphorothioates to the bases (Kurreck, 2003).
Even with an appropriate AS (or control) sequence, the delivery of said oligonucleotides must also promote the internalization and uptake. To ensure efficient cellular uptake and sustained gene inhibition, receptor-mediated endocytosis or transfection reagents can be employed to encapsulate nucleic acids (Kurreck, 2003; Achenbach, Brunner, & Heermeier, 2003). This will ensure the ability of AS sequences to cross lipid membranes (Achenbach, Brunner, & Heermeier, 2003). Interestingly, efficient nucleic acid 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 Specific Aims

      2.1 To Determine the effects of Synapsin II knockdown on the attentional performance of Rat as evaluated by the 5-CSRTT.

      The purpose of this study is to investigate the effects of synapsin II knockdown on attentional performance as measured by the 5-CSRTT. In recent years an emphasis has been placed on the cognitive impairments associated with SCZ. It has been determined that not only are these impairments a core feature of the disease, but they tend to precede the onset of psychosis while not responding to APD treatment (Young et al., 2012; Young et al., 2009). Due to these reasons, the National Institute of Mental Health (NIMH) created a specialized initiative known as MATRICS (measurement and treatment research to improve cognition in Schizophrenia), with the purpose of research and development of therapies concerning the cognitive (and some negative) effects of SCZ (Marder and Fenton, 2004). Attention/vigilance has been determined as one of seven core cognitive domains by MATRICS. Young described attention as:

"...the ability to allocate and sustain the focus of cognitive resources on specific stimuli or information while ignoring or filtering other information." – (Young et al., 2012).

  In fact, this may be considered as a sort of modulator/mediator of all other cognitive domains showcasing an importance for further study.

      The five choice serial reaction time task (5-CSRTT) is a paradigm designed to assess attentional performance in rodents (Young et al., 2012). This task has been described as a rodent-based amalgamation of Leonard's five choice task (subjects must tap one of five randomly illuminated light bulbs as quickly as possible) and the continuous performance task (CPT) (similar to Leonard's task, but the subject must only respond to certain pre-determined stimuli, while ignoring other stimuli) in clinical populations (Nuechterlein and Dawsom, 1984). It was found that SCZ patients performed worse on the CPT task than healthy controls, while imaging studies determined that the frontal cortex was responsible for "normal" performance (Young et al., 2012). It has also been determined that established APDs do not fully restore cognitive ability or performance in CPT (Young et al., 2012). Therefore, it is of experimental value to determine the role of synapsin II in the attentional deficits associated with SCZ, and whether a reduction in synapsin II protein levels could manifest such deficits.

      The 5-CSRTT can be utilized in rodents to determine various facets of attention. Various other experimental measures can also be collected via the chambers used. In order to determine attentional ability one may be primarily concerned with accuracy of responses; however, the other measures; latency of response, omission totals, perseverative responses, etc. can help to further the understanding of the subject's behaviour (attention versus motivation, etc.). Chronic amphetamine treatment can cause a reduction in accuracy when compared to control animals, while chronic administration of clozapine has been shown to ameliorate said reductions (Amitai and Markou, 2010; Amitai, 2007). Similarly, chronic amphetamine treatment also caused a reduced accuracy, which can also be reversed via the D1R agonist - SKF38393 (Fletcher et al., 2007).

AIM #1:The first aim of this study is to determine if synapsin II knockdown in the mPFC of adult male rats causes attentional dysfunction that can be measured with the 5-CSRTT.

Due to the extent of PFC involvement in the CPT for clinical populations, as well as the importance of synapsin II in cortical synapse function, it is hypothesized here that a knockdown of mPFC synapsin II will result in lower accuracy scores. In addition to the reduced accuracy, it is hypothesized that there may be significant differences in additional measures assessed by the 5-CSRTT.

2.2 To determine the effects of Synapsin II knockdown on metabolic activity in various brain regions utilizing fMRI/PET imaging.

In the past, multiple studies have shown that SCZ can cause a reduction in cortical metabolism during cognitive activation when compared to healthy controls (Floresco et al., 2006; Wolf et al., 2002). Both clinical and animal studies have yielded similar results when fluorine-18 fluorodeoxyglucose ([18F]FDG)(radiolabeled tracer) is utilized in concordance with PET imaging in regards to SCZ. The radiolabeled FDG acts as a measure of cerebral glucose utilization, a measure directly correlated with neuronal activity (Wolf et al., 2002). Clinical studies have shown that patients with SCZ present with metabolic hypofunction in various brain regions, specifically the PFC (Wolf et al., 2002; Birrell and Brown, 2000). As mentioned earlier, pre-clinical animal work has results which mimic the clinical results. It has been shown that chronic PCP sensitization in rats resulted in reduced metabolism in brain regions, while the NMDA non-competitive antagonist MK-801 resulted in regions of hyperfunctioning metabolism (Daya et al., 2014; Rodefer et al., 2008).

AIM #1: The aim for this study is to determine whether synapsin II knock-down in the adult male rat mPFC resulted in hyper- or hypo- metabolic functioning at the site of infusion.
It is hypothesized here that the immediate site of infusion (at the very least) would present as hypometabolic following cortical activation and the subsequent PET scan.

AIM #2: To determine if the performance on the radial maze can be indicative of the degree of metabolic changes.
Additionally, we would like to determine if results from the scans could be correlated to performance on the radial 8-arm maze (cortical stimulating task). It was postulated that the level of hypofunction would be a reasonable predictor of performance, or vice versa.

3.0 Methods
3.1 Synapsin II Knockdown
    3.1.2 Oligonucleotide Design & Prep
AS and MM sequences were determined by via NCBI BLAST tools available online. Once a specific sequence was determined, deoxyoligonucleotide sequence was synthesized via Integrated DNA Technologies (IDT) (Coralville, Iowa, USA). All nucleotides were capped with phosphorothioates to prevent degradation via nucleases. Oligonucleotides were dissolved in nuclease free water (NFH2O) to a final concentration of 1uM.

3.1.3 Animal Surgery/Oligonucleotide Delivery

Following 5 choice serial reaction time task (5-CSRTT) training, stereotaxic surgery was performed on the animals. Surgical procedure follows that of Dyck et al. (2007) previously performed in our lab. Rats were anesthetized with isoflurane and bilateral, stainless-steel cannulae were inserted into the mPFC according to the following coordinates from bregma: AP +3.5, ML ±2.0, and DV -3.5 (Dyck, 2012; Dyck, 2011; Dyck, 2007). Two-week Alzet osmotic mini-pumps (HRS Scientific, Montreal, QC) were filled with 5nM of solution of the respective treatments. Cohort 1 (5-CSRTT % primary imaging): Group A (n=8) was infused with artificial cerebral spinal fluid (aCSF); Group B (n=7) was infused with synapsin II antisense deoxyoligonucleotides; and Group C (n=7) was infused with a mismatch oligonucleotide sequence. Cohort 2 (secondary imaging & Maze): Group D (n=5) was infused with aCSF; Group E (n=6) was infused with synapsin II AS oligonucleotide; and Group F (n=6) was infused with a mismatch synapsin II oligonucleotide.
Rats were left to recover over the next 4 days prior resuming behavioural testing. The total time of infusion lasted approximately 14 days.

3.2 Rodent Behavioral Testing
3.2.1 5-CSRTT

5-CSRTT training and testing was completed in a dimly lit room, with animals being housed on a reverse light cycle. Both behavioural testing and training were completed in operant boxes from Med Associates (St. Albans, VT) measuring 33m x 31cm x 29cm. The chamber consists of an array of 5 apertures (light and detector), located opposite a single reward aperture. Similar to the array openings, the reward compartment has a photo detector spanning the entrance, but acts as the final receiving place for food pellets dropped from an attached pellet hopper. Overhead there is also a light source used to convey an adverse stimulus. The entire operant chamber is subsequently placed inside a large wooden test cabinet to reduce confounding variability from outside stimuli. 45mg Bioserv Dustless Precision Pellets (no flavour) obtained via Cedar Lane (Burlington, ON) were used for both testing and training. Chamber units were controlled by a desktop computer running Med-PC IV software.

For habituation purposes, rats were allowed to freely roam the chambers with all aperture lights lit and food pellets in each opening (usually 1-2 days). Once habituated, 5-CSRTT training began. Training began at training stage 1 (T1) and progressed to training stage 12 (T12), at the speed of rat learning (~4-8 weeks) (Bari et al. 2008). Criterion to pass each stage consisted of a minimum of 50 correct responses, with an accuracy of 80%, and an omission rate below 20%. Regardless of intensity level, each 5-CSRTT session lasted for a maximum of either 100 trials or a maximum of 30 minutes.

Upon initiation of a session, a single food pellet was dropped into the reward compartment with an LED light concurrently lit. A break in the reward sensor by a nose poke indicates pellet retrieval and initiates trial #1. Following a set inter-trial interval (ITI), one of the five array compartments becomes illuminated. This compartment stays lit for a set period of time, also known as the stimulus duration (SD). The rat can provide a response during the SD, or for a specified time after, known as the limited hold (LH). A correct response occurs when the array compartment, lit via the LED, registers a response poke. Upon this correct response, the pellet hopper rotates to release a single pellet into the lit reward compartment. Once the food pellet is

recovered and detector plane broken, trial #2 is initiated. An incorrect response occurs when a poke response is registered by an array box that is not lit, during either the SD or LH. This incorrect response causes the overhead cage light to be illuminated for a timeout period (TO) of 5 sec. The next trial is initiated following the TO period. An omissive response occurs when no nose poke is recorded by any of the 5 array boxes throughout the SD and LH. When this occurs the overhead light is again illuminated for a 5 sec TO period. A premature response occurs when a nose poke is registered by an array during the dark ITI period prior to an array box being illuminated. This again causes a 5 sec TO period. TO period was reversed from a dark period to a well-lit (bright) period to correspond to the reverse light cycle. During the training and testing period, data is collected based on response type and response latency.

Animals begin training at T1, with the following parameters: 30 sec SD, 2 sec ITI, and 30 sec LH. Parameters grew progressively more difficult, upon graduation from a stage, until rats reached a final stage (T12) with parameters: 0.5 sec SD, 5 sec ITI, and 5 sec LH (Bari 2008). Following a stable performance on the final stage (T12), rats have shown a stable understanding of the paradigm and are thus suitable for surgery and testing. Rats then underwent surgery to induce the respective treatments as previously described (Dyck 2010, 2006, 2007, 2011). Following recovery from surgery, rats were tested in the 5-CSRTT in a fashion similar to that of training. Various stages and parameters were used during this testing phase to evaluate performance under varying SD, ITI, and LH, in order to probe for the effects of treatment on measured cognitive parameters. All rats were tested under the same parameters for any single session. Rats completed the 5-CSRTT five days a week, once (sometimes twice during training) per day. During the course of the study, rats were also food restricted to 2% body weight/day (~15-16g), and fed only following completion of their daily testing. This was assumed to maintain motivation with a food reward.

3.2.2 PPI

The pre-pulse inhibition test is conducted utilizing the Startle Response System designed by SR Labs (Sand Diego Instruments, Sand Deigo, CA). Details of the behavioural paradigm has been previously cited by our lab (Dyck et al., 2012; 2011; 2007). Baseline pre-pulse inhibition was tested prior surgery to ensure that all animals are equally sensitive in response to the pulse or pre-pulse presented. These behavioural controls are necessary caveats in order to attain interpretable behavioral findings. Rats were also subject to pre-pulse inhibition testing post-surgery (day 14) to ensure efficacy of treatment. PPI sessions included a 5 min habituation to the restraint tube, immediately followed by a 15 min testing portion. Acoustic parameters were set as follows: startle pulse - 100dB for 40ms; prepulse - 67dB, 70dB or 73dB for 20ms; no stimulus - white noise.

3.2.3 Social Interaction

Details of the behavioural paradigm has been previously cited by our lab (Dyck 2012, 2011, 2007). An hour prior to testing, rats are habituated to the room. Two unfamiliar rats from the same treatment group are placed into a black polyvinyl open box (100 cm x 100 cm x 40 cm) at the same time. A ceiling-mounted video camera above the arena recorded the interaction for a 5-minute period. Videos were then analyzed for total number of active, passive, and total time of interaction for each rat. Active interaction is defined as purposefully sniffing, following, crawling, grooming, or aggressive behavior. Close proximity without any of the previously mentioned

behavior is considered passive interaction. Baseline social interaction was also performed prior surgical treatment, and each combination pair of rats was only used once. Social interaction was again performed post-surgery (day 15) to ensure efficacy of treatment.

3.2.4 Locomotor

Locomotor testing was conducted in specialized AccuScan chambers (AccuScan Instruments, Columbus, OH), controlled by AccuScan software via the connected computer. Details of the behavioural paradigm has been previously cited by our lab (Dyck et al., 2009, 2011, 2012). Baseline locomotor activity was measured prior to training and surgery to ensure that there are no subtle underlying basal differences in walking speed and distance travelled between groups. Locomotor activity was tested on post-surgery (day 15) to ensure efficacy of treatment.

3.3.0 Imaging Study
3.3.1 Brain Imaging

In collaboration with the McMaster Centre for Pre-clinical and Translational Imaging, PET and computerized tomography (CT) imaging was performed in the first cohort of adult rats (receiving fully phosphorothioated oligonucleotide sequences). Local cerebral glucose utilization values were obtained by measuring glucose uptake in the prefrontal cortex. The PFC was selected as the primary region of interest due to irregular metabolic function observed in the frontal cortex of schizophrenic patients (Floresco et al. 2006; Wolf et al. 2002). Animals from the sense and AS groups (n = 4/group) were selected for imaging. The rodents were fasted for 12 hours prior to imaging and lightly anesthetized using 1.5% isoflurane (Pharmaceutical Partners of Canada Inc) for administration of 500 µCi of [18F]FDG in 400 µL of saline via tail vein injection. Immediately following the injection and recovery from anesthetic, the rats were transferred to the 8-ARM to cerebrally stimulate the animal. All animals were previously habituated and trained to the 8-ARM paradigm for three weeks prior (Section 3.7.8). Following completion of the cognitive task, the rat was allowed to rest in its home cage until 30 minutes had elapsed since the [18F]FDG injection. After the time had elapsed, the rat was anesthetized using 2% isoflurane and positioned in a bio-containment chamber designed for imaging. Each animal underwent a 15 minute static emission PET scan followed by a 5 minute high resolution CT acquisition. The animals remained anesthetized throughout the imaging process. Imaging was conducted in a Philips Mosaic dedicated Animal PET system (Philips Medical Systems, Cleveland, OH, USA), and Gamma-Medica Ideas X-SPECT (Gamma-Medica Ideas, NorthRidge, California, USA).

3.3.2 8-Arm Radial Maze

Performance in the 8-ARM has been shown to be an indicator of spatial, working, and reference memory (Bardgett et al. 2008). The delayed win-shift procedure was utilized to assess working memory (Olton, 1987). The 8-ARM apparatus consisted of eight arms (70 cm long x 25.5 cm high x 12.5 cm wide), extending from an octagonal center platform (52 cm diameter), with guillotine doors placed at the center platform to prohibit entry into a given arm. A reward pellet (5-CSRTT pellet: Bioserv, via Cedarlane, Burlington ON) is placed at the end of each arm. Animals were trained to find food rewards in blocked and open arms of the maze. For seven consecutive days, rats performed a habituation phase wherein they had access to all eight arms for 10 minutes. Rats were then trained (approx. 14 days) for the win-shift version of the paradigm.

The win-shift format consists of two trials, a forced run and a free run respectively, separated by specific time delay. During the forced run, four arms of the maze are open and baited, while the other four arms were blocked/restricted. The doors to be blocked for the force run were randomly selected on a daily basis. Rats enter the maze via the centre and are tasked with searching for all four treats. Once all treats are consumed, or the expiration of time, the rat is removed from the maze and returned to its home-cage for a delay (either 1 or 15 min). Once the rat has finished the forced run, it is removed from the maze which is then cleaned via 75% ethanol. Next, the 4 closed-arms of the maze are now opened and baited. Following their delay, the rats are placed in the center of the maze for the free run. Rats then must navigate only the arms which are newly opened for the free run. The following data was recorded during the free run: 1) the number of correct responses during the first four arm choices post-delay (correct response considered when animal enters the correct arm and eats the treat), 2) the number of retroactive errors (i.e. visits to arms that were baited during the forced run), and 3) the number of revisits to arms that were baited during the free choice run. Retroactive errors and revisit errors are indicative of working and reference memory impairment (Olton 1987; Spieker et al. 2012; Bardgett et al. 2008). This protocol is a modified version of that from Bardgett et al. and Daya et al. (Bardgetts et al. 2008; Daya et al. 2014). Each rat is trained or tested in the maze 1 time daily (can be increased to twice daily to speed training). Testing on the 8-ARM paradigm was identical to the training sessions, and took place for eight consecutive days post-recovery from surgeries.

3.4 Biochemical Assays

3.4.1 Tissue Sacrifice, collection and preparation

Animals were anesthetized with 3.5% isoflurane (Pharmaceutical Partners of Canada Inc) prior to decapitation. Brains were then removed and the following brain regions were dissected over ice: mPFC, PFC, STR, and cerebellum (among others). The regions were stored at -80*C. Brain regions were homogenized via pestle in Tris-EDTA buffer (50 nM Tris, 1 nM EDTA, pH 7.4) with a Mini-C Protease Inhibitor Tablet (Roche, Mississauga, ON) before being sonicated for 5 seconds (on ice). A Bradford Assay was then run with Bio-Rad Protein Assay reagent (Bio-Rad, Mississauga, ON) and a CU-640 spectrophotometer (Beckman-Coulter, Mississauga, ON) to determine protein concentrations. Optical density (OD) of each sample was then measured at 595 nm. Samples which fell outside of the linear range of the purified bovine serum albumin (BSA) protein standard of 1.2 – 10.0 µg/mL were further diluted with the Tris-EDTA and a Mini-C tablet buffer before the OD was re-estimated. Samples were stored at -20*C until further use.

3.4.2 Western Blotting

Immunoblot assays were performed to verify knockdown of the synapsin II protein in the medial prefrontal cortical tissue of all three cohorts of rats. Additional immunoblotting was performed in the prefrontal cortical and striatal tissue. Western blotting for the synapsin II antibody was performed as previously described by Dyck et al. (2011). After determining sample concentrations, 15 µg of protein was separated by a 10% sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel electrophoresis. Protein was then transferred onto a 0.2 µm polyvinylidene fluoride (PVDF) membrane. Membranes were blocked for 1 hour with 5% milk buffer in Tris Buffered Saline (TBS) with Tween® 20 (TBS-T). Post blocking, blots were incubated with the synapsin II primary antibody (overnight, 4°C) and the respective secondary antibody (1.5-2 hours, room temperature) in TBS-T [Table 1]. Concurrent probing for the 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. Visualization of protein bands was achieved with enhanced chemiluminescence (ECL) reagents (Amersham Biosciences, Pittsburgh, PA, USA). Synapsin II membranes were exposed to film for 1, 5, 10 seconds, 30 seconds, 1 minute, and 2 minutes.

Table 1.

PRIMARY ANTIBODY		
	Antibody	Concentration
Synaptic Protein	Synapsin II	1:2500
Housekeeping	GAPDH	1:20,000
SECONDARY ANTIBODY		
	Antibody	Concentration
	Anti-rabbit	1:5000
	Anti-mouse	1:10,000

3.5.0 Data Analysis

       All above data was analyzed and graphed using GraphPad Prism 4.0 software (San Diego, CA, USA). Data was analysed using, the student's unpaired two-tailed t-test as both studies contained only 2 treatment groups. In the oligonucleotide study, data was analyzed using a one-way ANOVA with Tukey's post-hoc test. Statistical significance was defined as $p < 0.05$ (95% confidence levels). Outliers were identified using Grubb's test with a significance level of $\alpha \leq 0.05$.

 Prepulse Inhibition: PPI results analysis was performed similar to that described by Dyck et al., (2011). The formula: $\%PPI = \square - \square \ \square \times 100$, where S is the average response to a startle-pulse only trial, and P is the average startle response to a prepulse-startle pulse trial, was used to determine percent inhibition (%PPI). % PPI was calculated for each prepulse decibel level, and obtained between treatment groups (Dyck et al., 2011). Cohorts were either analyzed using the student's unpaired t-test or the one-way ANOVA, depending on the number of groups.

  Locomotor Activity: The total distance travelled (cm) by subjects was recorded and analyzed over a 180 minute period and over 150 minutes (minus the 30 minute habituation). Cohorts were analyzed using either a student's unpaired t-test or a one-way ANOVA depending on the number of groups.

  Social Interaction: Each rat in all treatment groups was assessed via the following parameters during a 10 minute time frame: 1) interaction (total time spent) (min), 2) number of interactions in total , 3) total number of active interactions, and 4) total number of passive interactions. The first 1-2 minutes were excluded to account for habituation. The number and length of the interactions were then analyzed for 5 minutes. Social interaction data was analyzed using the student's unpaired t-test to determine statistical significance.

 18F[FDG]: PET data was reconstructed using a quantitative 3D iterative reconstruction algorithm. CT data was using a Feldkamp filtered backprojection cone beam reconstruction algorithm. Prior to analyzing [18F]FDG uptake, our lab applied Amira 3D Software (Hillsboro, Oregon, USA) to process raw MRI images of a healthy brain, and convert them into 3D images which were then fused together to form 'labels' of various brain regions. Following creation of the labels, our PET and CT images were fused together and aligned with our existing MRI images using the software programs: FreeSurfer and FMRIB Software Library. Utilizing MatLab software (Natick, Massachusetts, USA), activity in the cortex was quantified by assessing [18F]FDG uptake relative to the control labels. Values were obtained for each sample and region, and were then analyzed for significance using a student's unpaired t-test.

4.0 Results
4.1 Behavioral Testing
    4.1.1 5-CSRTT
**Percent of Correct Responses:** The percent of correct responses is measured by the total number of correct responses divided by the sum of correct and incorrect responses, and is considered to be the main measure of attentional performance (Amitai et al., 2010). A significant effect of treatment was observed ($F_{2,349}$=45.34, $p<0.0001$), with Tukey's post hoc analysis revealing a

significant decrease in percentage of correct responses in synapsin II knockdown animals, when compared to both aCSF- and mismatch-treated control groups (p<0.001) [**Figure 5(a)**].

Percent of Omissions: Percent omissions were calculated in a fashion similar to the percent of correct responses. The treatment group had a significant effect on percentage of omissive responses ($F_{2,349}$=56.69, p<0.0001), with post hoc analysis showing a significant increase of omissions in synapsin II knockdown animals when compared to both aCSF- and mismatch-treated control groups (p<0.001) [**Figure 5(b)**].

Number of Premature responses: Premature responses are defined as those committed prior to the presentation of a cue light and can be interpreted as a measure of impulsivity. No significant difference was found between groups, and thus there was no effect of synapsin II knockdown on the number of premature responses committed ($F_{2,349}$=0.2867, p>0.1) [**Figure 5(d)**] .

Number of Perseverative Responses: Perseverative responses are defined as repeated responses made following a cue light response, prior to retrieving food pellet. This behavior has been determined to represent persistence and lack of cognitive flexibility by responding to a previously rewarding experience. Treatment was found to have a significant effect on the number of perseverative responses produced by rats ($F_{2,\ 348}$=7.142, p=0.0009). Our aCSF group committed significantly less perseverative responses when compared to both mismatch (p<0.05) and knockdown groups (p<0.01) [**Fig. 5 (e)**]
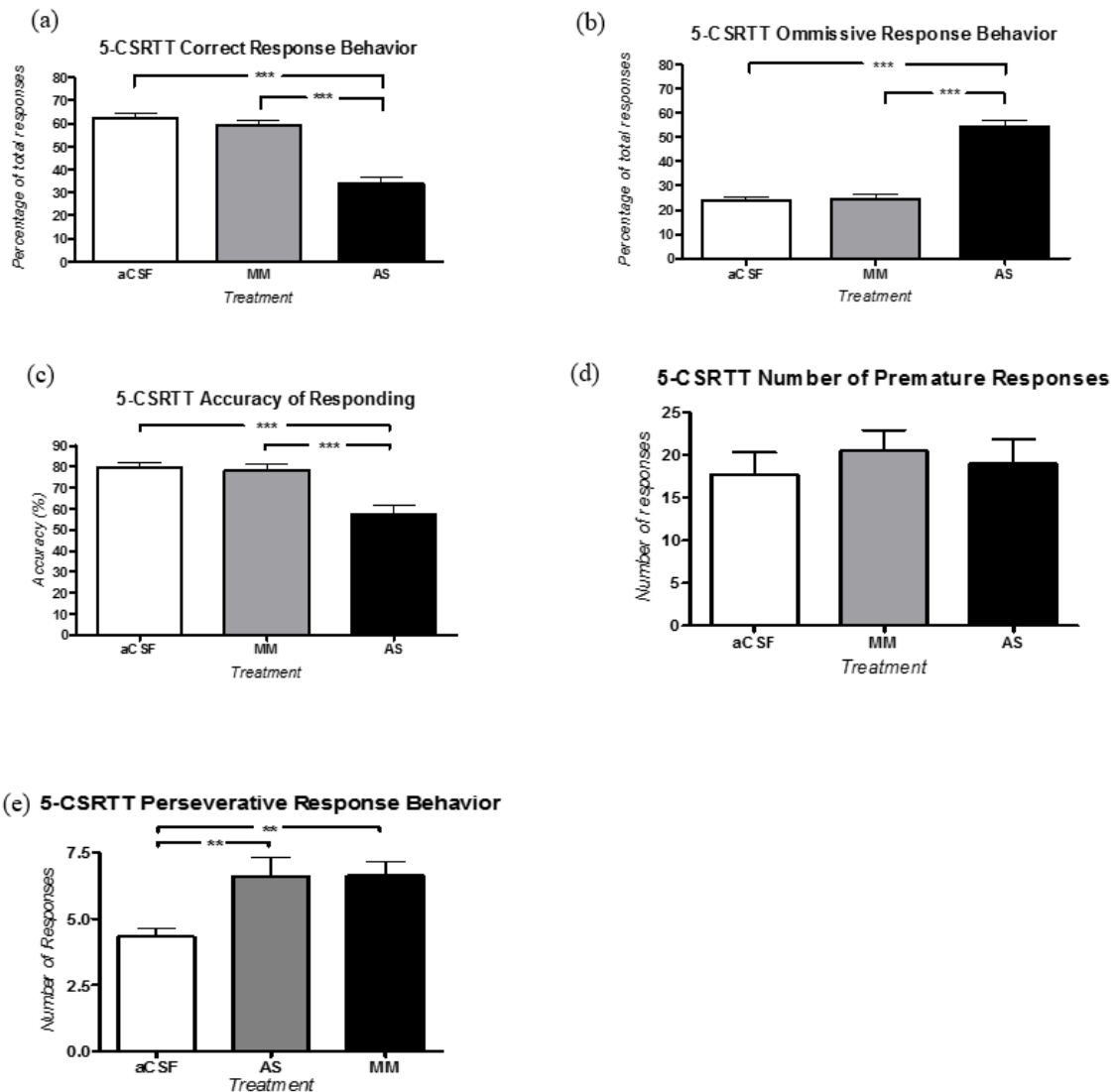
Figure 5. Behavioral effects of mPFC knockdown of synapsin II on the 5-CSRTT. Treatment groups include mPFC infusion of synapsin II ADON(n=7), and are compared to aCSF (n=8) and mismatch oligonucleotide control (n=7) groups. A: Percentage of correct responses was significantly decreased in the SynII ADON group compared to controls ***P<0.001. B: Omissive response percentage was significantly increased in the SynII ADON group compared to controls. ***P<0.001. C: Accuracy of responding (correct/total # of trials) was significantly reduced in SynII ADON group compared to controls. ***P<0.001. D: Number of premature responses was not significantly different across groups. E: SynII ADON group and mismatch controls performed significantly more pervasive responses than did the aCSF treated group. **P<0.01.

Latency to correct: Latency to correct is the latent time (s) between the presentation of a response cue to when a response is registered. Treatment had a significant effect on latency to correct time ($F_{2,349}$=7.981, p=0.0004); with Tukey's post hoc analysis revealing a significant increase in response time in the synapsin II knockdown group compared to both aCSF-treated (p<0.001) as well as mismatch-treated (p<0.01) control groups [**Figure 6(a)**].

Latency to reward: Latency to reward is the latent time (s) between the presentation of a reward pellet and when the reward is retrieved. This parameter is often considered a measure of motivation. A significant effect on latency to reward time ($F_{2, 329}$=5.695, p=0.0037) was determined with respect to treatment. Tukey's post hoc analysis revealed a significant increase in response time in the synapsin II knock-down group compared to both aCSF-treated (p<0.01) as well as mismatch-treated (p<0.05) control groups [**Figure 6(b)**] .
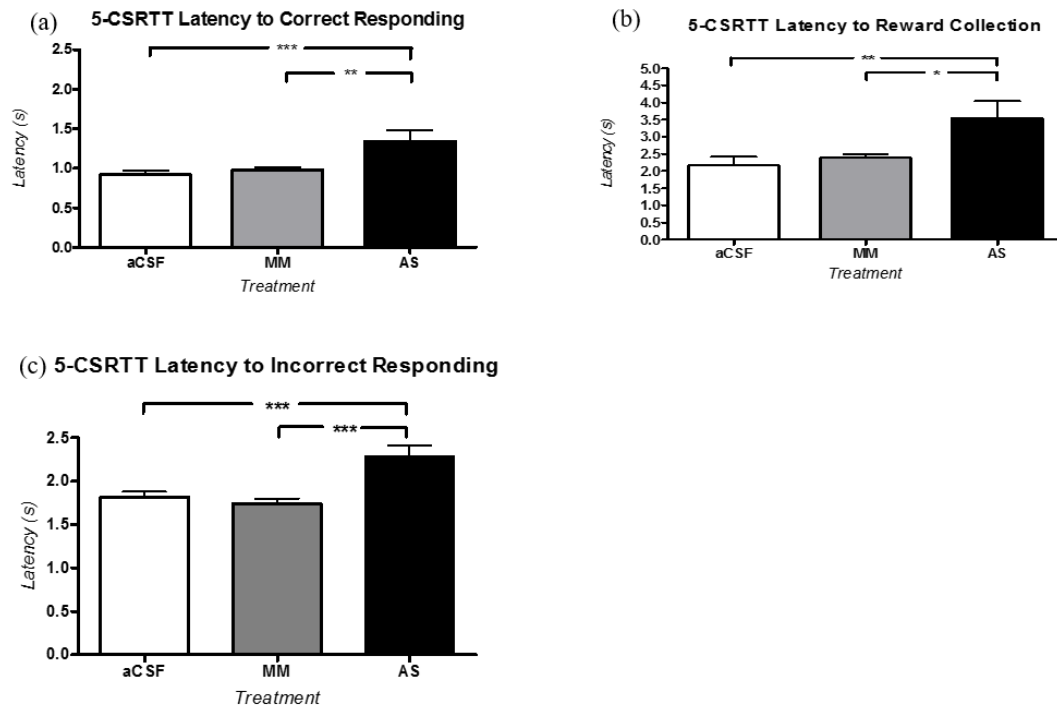
Figure 6. Effects of mPFC knockdown of Synapsin II on latency to various responses on the 5-CSRTT. Treatment groups are the same as discussed above. A: SynII KD animals take significantly longer to respond to the correct visual stimulus than control groups. **P<0.01, ***P<0.001. B: SynII KD rats took significantly longer to retrieve the food reward pellet upon correct responses. *P<0.05, **P<0.01.

Number of Head Entries (Nose-Poke Responses): Total number of nose-pokes is also another measure of a motivation. As indicated in **[Figure 7(c)]**, Treatment did not affect the number of nose-pokes committed.
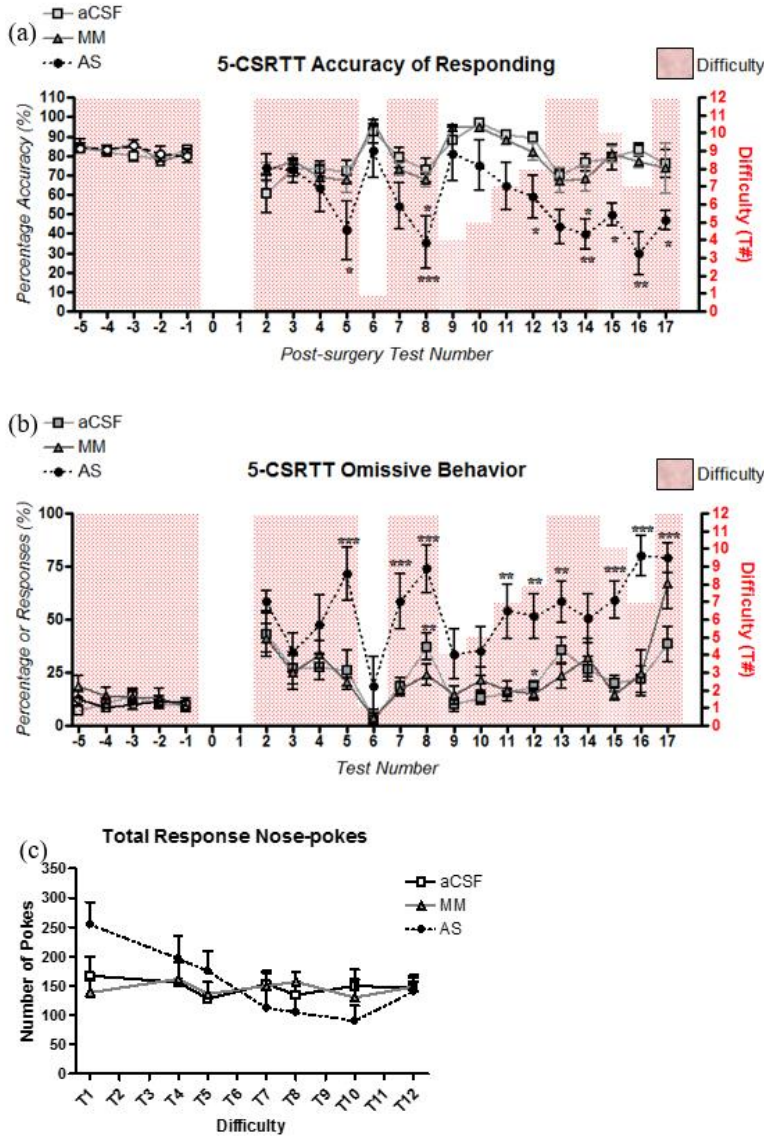
Figure 7. Behavioral performance of mPFC SynII knockdown compared to control groups on the 5-CSRTT as a function of task difficulty. Treatment groups are the same as discussed previously (same cohort). Responses are indicated over 17-post surgical tests. Test numbers <0 refer to baseline results obtained prior to surgical cannulation. Days 0 & 1 represent the day of surgery and recovery respectively. Pos surgical data collection began on test 2, and proceed for approx. 14 days. Shaded bars indicate difficulty of corresponding task. A: SynII KD rats had a significantly lower accuracy of responding than control groups. *P<0.05, **P<0.01, ***P<0.001. B: SynII KD rats performed significantly more omissions than both control groups. *P<0.05, *P<0.01, ***P<0.001. There was no effect of treatment or difficulty determined with respect to total nose-pokes.

4.1.2 Locomotor

Locomotor activity is used to determine deficits in motor control or total locomotion. Differing treatments induced a significant increase in locomotor activity ($F_{2,20}=3.620$, $p=0.045$), with Tukey's post hoc analysis revealing a difference between aCSF and knockdown groups ($p<0.05$). No significant difference was observed between synapsin II knock-down and mismatch groups [**Figure 8(a)**].

4.1.3 PPI

Pre-pulse inhibition is a task used to measure the intensity of startle response, reflecting sensorimotor gating of subjects. An effect of treatment was determined to induced a significant decrease in percent inhibition ($F_{2,16}=5.956$, $p=0.0117$), when comparing the knockdown group to both control aCSF ($p<0.01$) and mismatch groups ($p<0.05$) [**Figure 8(b)**].

4.1.4 Social Interaction:

Social interaction is the quantitative time measure of paired subject interaction and can be used to determine social withdrawal.   Treatment induced a significant decrease in total time interaction ($F_{2,19}=4.29$, $p=0.029$) when comparing knockdown to aCSF treated rats ($p<0.05$) [**Figure 8(c)**].

4.1.5 Biochemical Assay

Western blot gel electrophoresis is used to determine protein expression levels of synapsin II in harvested brain tissues. Treatment with antisense oligonucleotide caused a significant reduction of Synapsin IIa in the mPFC compared to aCSF treated rats ($p<0.005$) [**Figure 8(d)**]. Similarly, treatment with AS also caused a significant reduction of Synapsin IIb in the rat mPFC compared to aCSF treated controls ($p<0.005$) [**Figure 8(e)**].
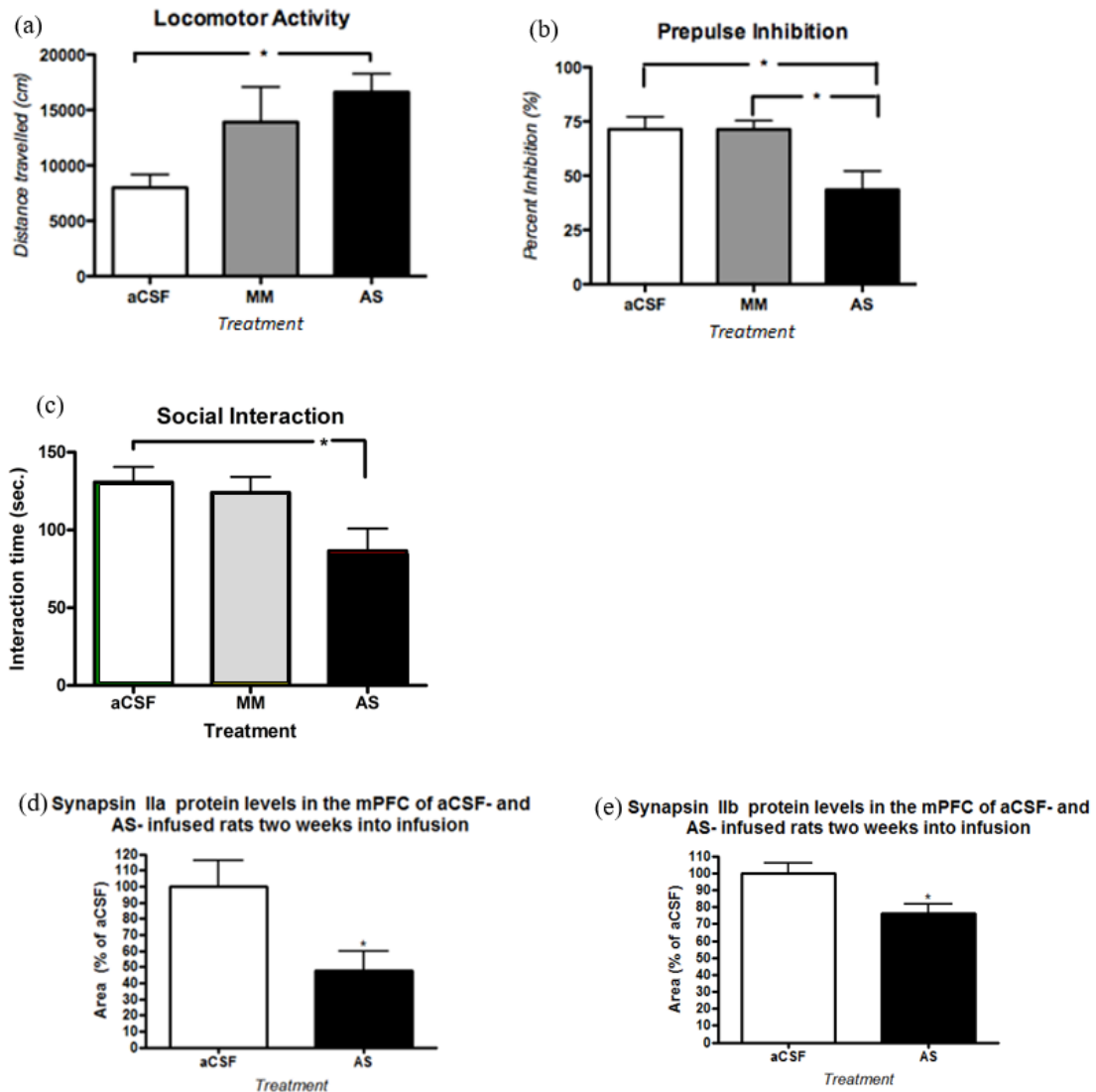
Figure 8: Behavioural effects of medial prefrontal cortex (mPFC) infusions of synapsin II antisense (AS) deoxynucleotide (n=7) when compared to aCSF (n=8) and mismatch oligonucleotide treated control groups (n=7), unless otherwise indicated. **(a)** Locomotor activity task indicate that synapsin II knockdown rats are significantly more active than aCSF controls. *P<0.05. **(b)** Percent startle inhibition to pulse (120dB) in synapsin II knockdown rats (n=6) is significantly lower when compared to aCSF (n=8) and mismatch oligonucleotide (n=7) control groups when presented with prepulse (77dB). *P<0.05. **(c)** Synapsin II knockdown rats are significantly less interactive than those in the control groups. *P<0.05. **(d)** Synapsin IIa was significantly decreased in the mPFC of AS-treated as compared to aCSF-treated groups. *P<0.05. **(e)** Synapsin IIb was significantly decreased in the mPFC of AS-treated as compared to aCSF-treated groups. *P<0.05.

4.2 Brain Imaging
4.2.1 [18F]FDG Uptake
Mean uptake value of [F18]FDG, as represented by PET readings, can be used as a measure of metabolic activity for brain regions in question. This information pertains to SCZ as it will be used as a measure of neuronal activity: increased activity would require an increase in energy, provided by an increase in metabolism. Therefore, levels of FDG will be indicative of neuronal function. Treatment with AS caused a significant increase in mean [F18]FDG compared to mismatch control animals ($F_{2,11.4} = 0.1242$, $p < 0.05$) . A two-way ANOVA utilizing Tukey's Post-Hoc analysis also revealed a significant effect due to brain region (which can be expected).
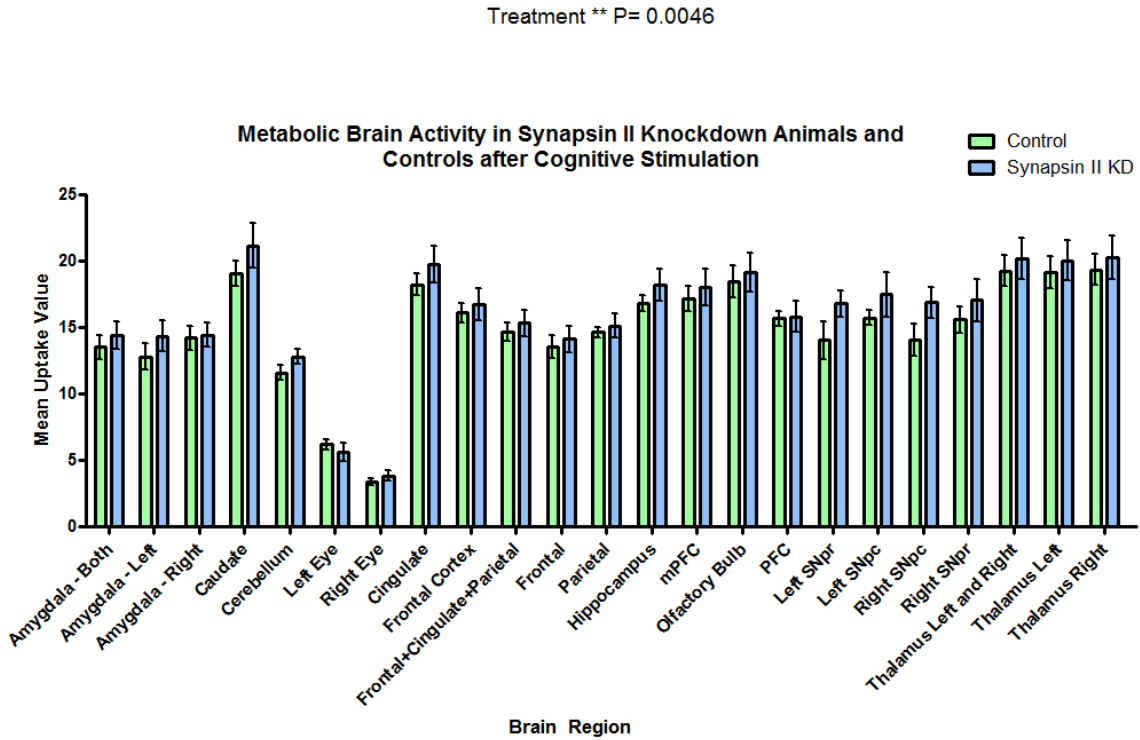
Figure 9. [18F]FDG uptake levels as determined via PET. Subjects are challenged via 8-arm radial maze 30 min prior to PET scan. Brain regions are indicated on x-axis with groups distinguished via colour. Statistical analysis revealed a significant effect due to treatment (P<0.005). A significant effect of region was also determined (P<0.001). Results indicate an increase in brain metabolism (activity) due to synapsin II AS treatment.

4.2.2 8-arm Radial Maze

Radial 8-arm maze was utilized to not only determine a measure of cognitive function, but also as a frontal activator for the above imaging experiments. Results show that treatment with AS caused a significant increase in latency to complete task ($F_{2,11}=7.853$, $p<0.05$) [**Figure 10(a)**] when compared to control animals. Conversely, no significant increase in errors types, both active and retroactive, were observed in AS rats compared to control rats ($F_{2,11}=8.4$, $p>0.05$) [**Figure 10(b)**] and ($F_{2,11}=3.773$, $p>0.05$) [**Figure 10(c)**] respectively.
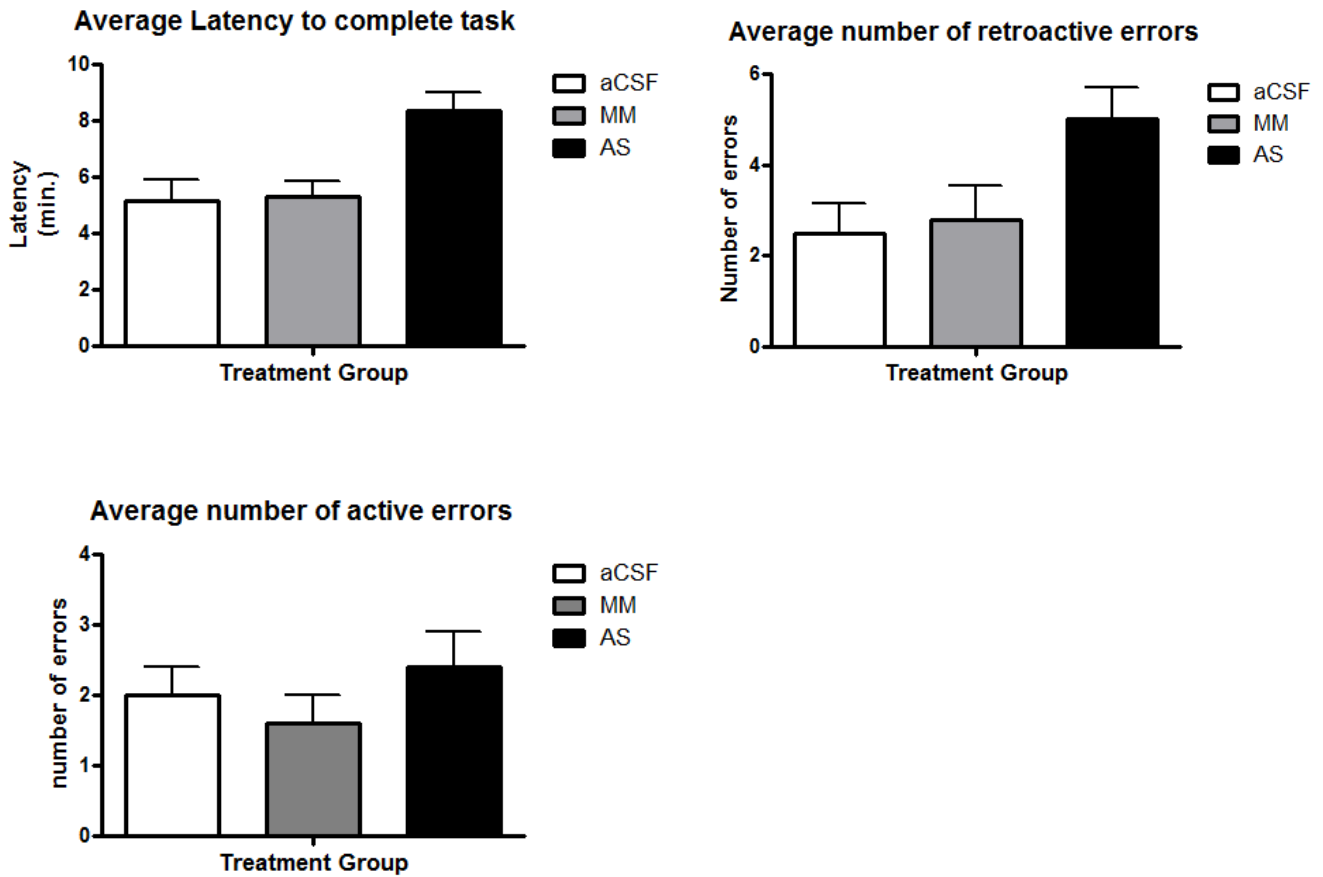
Figure 10. 8-arm radial maze results when used as a cognitive activator for PET scans. A: SynII KD rats took significantly longer to complete the task than did control groups (*P<0.05). No significant difference was observed in errors (either type) between treatment groups.
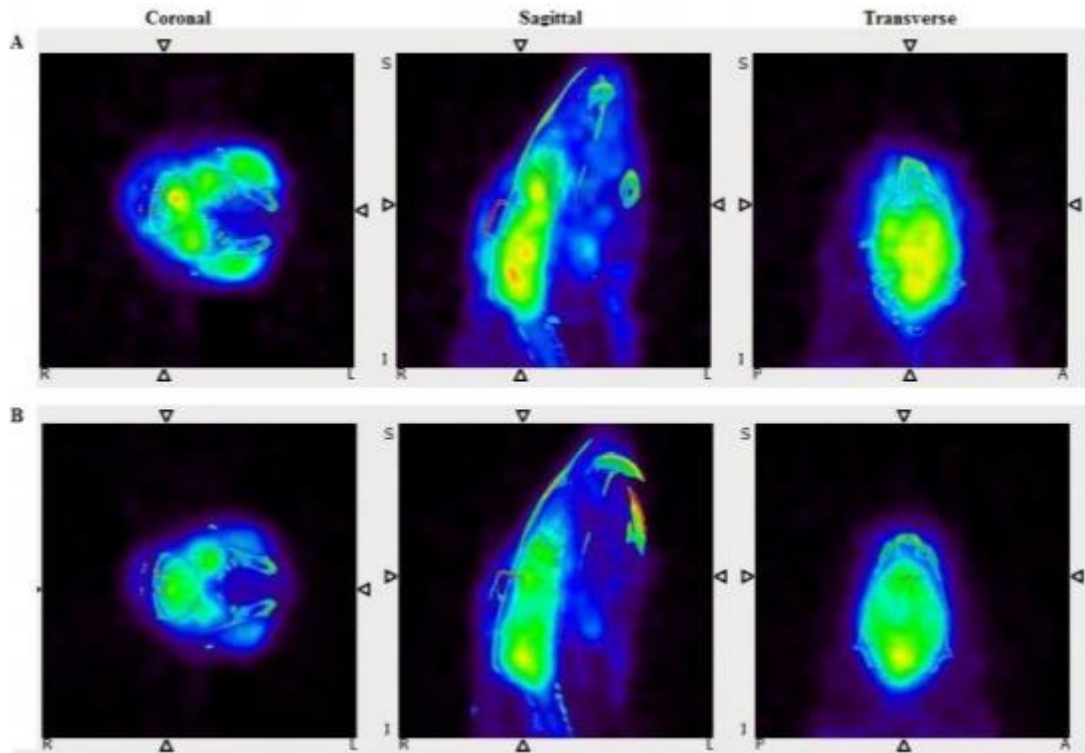
[**Figure 11**] [18F]FDG uptake in the adult rat following cognitive activation via the 8-arm radial maze paradigm. Guide cannula can be seen implanted at location discuss earlier in methods. A. [18F]FDG uptake in a rat bilaterally infused synapsin II antisense sequence. B. [18F]FDG uptake in rat bilaterally infused with synapsin II mismatch solution. Image is a simple representation of hyperfrontality observed in this experiment. Rats pictured were not subjects in this exact study.

5.0 Discussion

Presynaptic function and plasticity is an important aspect to modulating learning and memory in mammals (Kushner et al., 2005). The proteins involved in the regulation of neurotransmission, or in the docking of synaptic vesicles are obvious candidates to affect cognition. Impairments in synaptic neurotransmission and plasticity have been shown to cause the development of neuropsychiatric disorders (Citri, 2008). In fact, evidence have shown the integral role of the synaptic proteins such as the SNARE complex, SNAP-25, synapsins, and other associated synaptic proteins in the pathogenesis of schizophrenia (Johnson, 2008; Waites, 2011; Mirnics et al., 2000) Synapsins play crucial roles in the formation and maintenance of synapses, and a dysfunction in the expression of these synapsins could influence cognitive behaviours.

Synapsin II, in particular, has been strongly implicated in the pathogenesis and etiology of schizophrenia (specifically the synaptic theory of schizophrenia). Synapsin II is thought to be particularly essential for synaptic trafficking during repetitive stimulation and long-term regulation of glutamatergic and GABAergic vesicle reserve pools (Waites et al., 2011; Matus-Leibovitch, 1997). Glutamatergic dysregulation, as a result of synapsin II reduction, in the prefrontal cortex can subsequently alter GABA and dopamine release in the cortical and subcortical regions of the brain through local circuits and long loop pathways, resulting in neurochemical changes, which are characteristic of schizophrenia (Moghaddam et al., 2002; Stone et al., 2007; Marsman 2011). Glutamate hypofunction has also been shown in studies to lead to behavioural and cognitive impairments closely representing symptoms of schizophrenia (Goff et al., 2001). As such, it is conceivable that the knockdown of synapsin II in the prefrontal cortex can lead to the production of the cognitive deficits measured by the 5-CSRTT as a result of hypoglutamatergic neurotransmission in the prefrontal cortex. Cognitive deficits seen both in animal models of, and patients with, schizophrenia are also likely a result of defective information processing in a number of brain regions and systems intimately involved with each other.

Results from our experiment have indicated that selective knockdown of synapsin II in the mPFC resulted in disturbances in 5-CSRTT performance, pre-pulse inhibition, locomotor activity, and social interaction, indicative of a schizophrenia-like phenotype. The 5-CSRTT was used to assess the role of synapsin II in rodent visual attention/vigilance. This paradigm is one of the most dynamic tests used to measure selective attention amongst rodents, and has been routinely used in various other animal models of schizophrenia to assess cognitive modalities including attention, response inhibition, cognitive flexibility, and processing speed (Brown et al., 2002; Amitai et al., 2010). Due to the complexity of cognition dysfunction in schizophrenia, it is necessary to access a broad range of cognitive domains when understanding and creating potential treatments for schizophrenia. The 5-CSRTT is, thus, an efficient method to access the various cognitive domains, while allowing room for various parameter manipulations to mimic schizophrenia-like disruptions (Amitai et al., 2010). Numerous studies have established the validity of this cognitive test to access both impairments and improvements in attentional performance across rodents and primates (Amitai et al., 2010).

Results presented showcase the effect of synapsin II knockdown on 5-CSRTT performance. It is crucial to consider all the various parameters measured in combination when interpreting results obtained from the 5-CSRTT (Amitai et al., 2010). Amitai et al. (2010) proposes that an increase in

both latency to reward and correct responses may be an indicator of one of three possibilities: namely a motivational problem, cognitive inflexibility, and/or deficits in motor control. Percent accuracy of the rats in the synapsin II knockdown group was significantly lower than aCSF- and mismatch- treated control groups. This can be attributed to the schizophrenic like deficits in cognition and/or motivation. When the attentional load was increased (ie. shorter visual presentation of stimulus duration) however, accuracy decreased across all groups, with greater deficits in the synapsin II knockdown group, indicating impaired attention is the cause of disrupted performance. Synapsin II knockdown rats made significantly more preservative responses than aCSF-treated rats, and may indicate cognitive inflexibility leading to compulsivity (Chamberlain et al., 2006). Looking at these parameters alone would suggest cognitive deficits rather than motivational deficits leading to 5-CSRTT impairment. However, rats in the knockdown group also committed significantly higher percent omissions, which tends toward a lack of motivation (Amitai et al., 2010). The same group also had significantly longer response times to both the stimulus and the reward, which again tend toward a motivational deficit (Semenova and Markou, 2007; Carli and Semanin, 1992). Considering the preceding two parameters alone, it would suggest that a motivational deficit is responsible for the behavioural abnormalities observed in the 5-CSRTT. That said, the synapsin II knockdown group also performed an equal number of head entries and premature responses to that of the control groups, even averaging slightly higher in the number of premature responses than the aCSF-treated controls. The similar total number of nose pokes and premature responses across treated groups indicates all groups are equally motivated, thus ruling out motivation, or the lack of, as a factor of behavior.Our data also shows a significant increase in locomotor activity in the knockdown group [**Figure 8(a)**], indicating that deficits in motor control are not the likely cause of poor 5-CSRTT performance. These measures further suggest that poor 5-CSRTT performance caused by the knockdown of synapsin II in the mPFC is due to cognitive inflexibility rather than deficits in motivation and/or motor control.

Translational behavior experiments, such as this, often suffer from various limitations; to which this experiment was no exception. For instance, some researchers argue that the frontal cortex of the primate supports a number of higher order behavioural complexities that may likely not be found in the rodent (Birrell et al., 2000). Several behavioural studies have, however, shown that the medial prefrontal cortex of the rodent mediates higher cognitive processes such as attention and executive function (Birrell et al., 2000). The rodent medial prefrontal cortex has been found to be homologous to and bears functional, rather than anatomical, similarity to the primate (dorso)lateral prefrontal cortex (Birrell et al., 2000) More importantly, most of what we know today about the human disease of schizophrenia and the circuitry implicated is based from research on nonhuman mammals (Frankle et al.,2003).As such, results from this study implicating the knockdown of synapsin II in the medial prefrontal cortex of the rat is essential to our understanding of schizophrenia, and closely represents the decreased synapsin II levels in the dorsolateral prefrontal cortex of the patient population with schizophrenia.

This behavioural task (5-CSRTT) is not without limitations. The pattern of rodent response in the 5-CSRTT has previously been criticized as being automatic or timed in nature. This drawback is present because of the manner in which stimuli are presented to the rat, with a regular ITI reinforced via the strict training regimen. Evidence has shown that rats can utilize cues

to respond at correct temporal intervals, rather than using the cues as a basis for response timing (Spratt et al., 2000). However, including variable ITIs or inhibition-required responses within the 5-CSRTT can counteract these biases and strengthen the 5-CSRTT as a cognitive paradigm. Another limitation is the translatability across species, specifically when drawing comparisons and conclusions from the rodent 5-CSRTT to that of the continuous performance task (CPT), a task often used to assess attention and vigilance in humans (Young et al., 2008 ; Day, 2008). This task involves a participant being able to discriminate between a signal trial and a noise trial, often in a serial fashion. Discrimination is determined via response patterns on the basis that the participant is to respond to a signal and withhold response during noise-only trials. Research shows those suffering with schizophrenia often performs poorly on this task; reflecting a deficit in attention or vigilance, which is often attributed to deficits in the core cognitive abilities (Cornblatt et al., 1994; Heinrichs et al., 1998). Despite the translation of results between the 5-CSRTT and CPT, several differences exist. An example is the presentation of a non-signal in the CPT wherein participants must withhold a response (Amitai et al., 2010). On the contrary, rats are required to respond each trial, which can lead to patterning and rhythmic responding in the 5-CSRTT (Spratt et al, 2000). To avoid the aforementioned patterned learning in animals, as well as to draw a closer comparison to the human CPT, a task known as the 5 choice continuous performance test (5-CCPT) has been developed. This task contains a condition in which all stimulus are active, at which point animals are required to withhold response in order to retrieve a food reward (Young, 2008). This measure allows one to determine a subjects level of inhibition to responding, making this a more relevant comparison to the CPT (Young et al., 2008). The 5-CCPT is, however, not without drawbacks. By adding an additional cue type (all lights lit requiring no response) and emphasizing on response inhibition, one may exaggerate other behaviors in comparison to 5-CSRTT. Exaggerated behaviours may include increased measures of mean correct latency, premature responses, and percentage omissions (Young et al., 2009).

Results indicate a correlation between synapsin II levels and poor performance on the 5-CSRTT. This study has taken into consideration both motivational and cognitive impairments as a result of synapsin II knockdown, which can be measured using this version of the paradigm. Future studies, however, will utilize either the 5-CCPT or a 5-CSRTT utilizing variable ITI; which will more stringently determine a subjects inhibition levels and allow for a more appropriate comparison of disease models across species. Detailed behavioral assessment of the symptoms of schizophrenia in the synapsin II knockdown rodent in this study will, accordingly, broaden our understanding into the pathophysiology of this debilitating disorder.

Taken together, the various behavioral commonalities between SCZ with BD and ASD, as well as common risk assessment between the synapsin II gene and the aforementioned diseases, a potential therapeutic value of synapsin II in mental health can be implicated (Greco et al., 2009). Aside from the information presented above, there are other hypotheses (past or current) which may benefit from studying synapsin II. With this in mind, continued work in synapsin II is both a current and future task.

Other pre-clinical animal models of SCZ are currently of interest with respect to synapsin II. Various pharmacological models of SCZ present differing symptom profiles making it difficult to accurately study the mechanism behind the etiology of SCZ. Currently the phencyclidine model of SCZ induces the most robust behavioural phenotypes, with preclinical animal models

displaying positive, negative, and cognitive symptoms. Similarly synapsin II knockout in adult rats also results in the development of the SCZ-like behaviors. Thus, there may exist an association between synapsin II expression and PCP induced deficits. Preliminary studies from our lab have found reduced levels of synapsin II expression in the in the mPFC of PCP-induced animal subjects (unpublished preliminary studies). These results, in addition to recent work addressing synapsin II dysfunction and its various resulting phenotypes (ASD, BD, etc.), continued experimentation with regards to synapsin II holds merit.

The eight-arm radial maze behavioural paradigm is used as an indicator of working and reference memory performance in murine animals (Bardgett et al., 2008). Patients suffering from SCZ have also shown difficulty navigating a virtual maze, this indicates an impairment in working memory. (Spieker et al., 2012). In this study, a delayed win-shift procedure of the 8-ARM was utilized to examine working and reference memory, as well as executive functioning. Control rats can successfully perform the 8-ARM paradigm, making few working and reference memory errors while correctly choosing to only enter those arms still containing a treat. Rats treated with synapsin II AS solution performed significantly worse in the 8- ARM, in comparison to the negative control, and sense treated rats. Although there was no difference between the two groups in terms of the number of correct choices, AS rats made significantly increased number of errors, both active and retroactive errors. Results therefore suggest that the knockdown of synapsin II within the medial prefrontal cortex interferes with working memory and executive function. An increase in retroactive and revisit errors infers deficits in reference and working memory,respectively.

Metabolic dysfunction has been commonly observed in patients with schizophrenia, however, this same assessment in live-rodent models of SCZ is less common (Daya et al., 2014). Cortical hyperfrontality is most commonly observed in the dorsolateral PFC of humans (an area analogous to the rodent mPFC) SCZ patients (Callicott et al., 2003; Daya et al., 2014).Therefore, we wanted to examine the effect of reduced levels of synapsin II in the mPFC on cortical activity. Following cortical activation using the eight-arm radial maze, PET/CT fused imaging demonstrated that AS infused rodents showed significant hyperfrontality in comparison to the negative control mismatch group. This finding suggests that mPFC synapsin plays a role in signalling/neurotransmission which can affect multiple domains of cognition. When used in live animals, the fused imaging method ensures accurate localization of functionally aberrant tissue, and allows for increased translatability, live animal analysis, and ease of measurement (Daya et al., 2014). The advantages of PET and CT fused imaging make this technique more favourable to identify abnormalities in cortical function in the live animal. To our knowledge, this is one of few studies to model cortical hypofrontality and/or hyperfrontality, using PET imaging, in the live rat.

A single area of concern lies with the latency of response in both the 5-CSRTT and 8-arm radial maze. Measures of response latency are often used to determine motivation within a specific task. In both of our cognitive paradigms we observed a significant increase in response latencies/completion times. This would imply that we may have a reduction in motivation. It may also imply a physical inability to complete the task in a rapid manner. However, the significantly increased levels of locomotor activity suggest that motor impairment was not the reason for these increased latencies.

6.0 Conclusion

Evidence substantiates a crucial role for synapsin II in the pathophysiology and therapeutic mechanisms for the treatment of SCZ. The empirical basis and experimental findings are summarized in [**Figure 12**]. 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 may subsequently promote the development of SCZ. The further understanding of synapsin II in these synaptic functions remains critical to unraveling the pathogenic mechanisms of SCZ, and may facilitate the production of novel and safer therapeutics for treatment of this, and other,debilitating disorders.
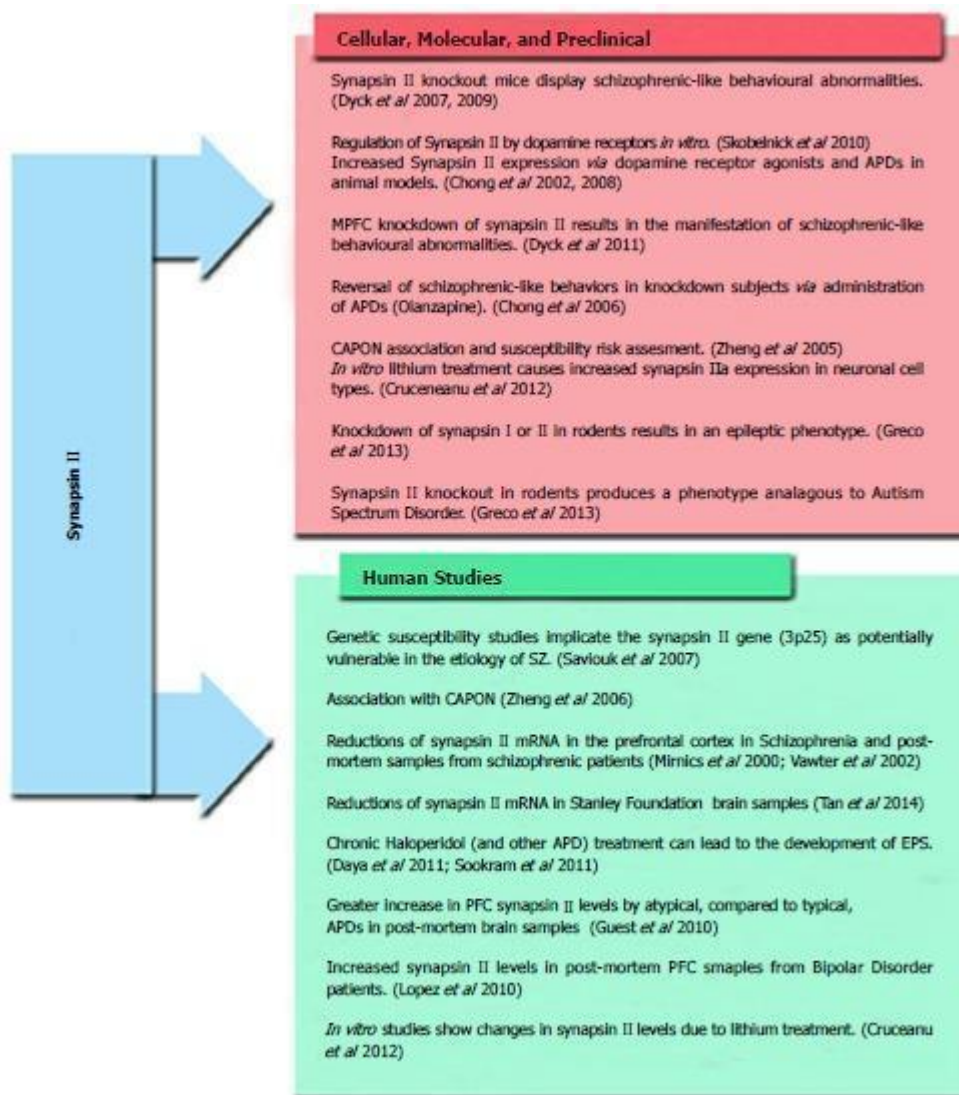
Figure 12. Empirical basis and experimental evidence suggesting the involvement of synapsin II in schizophrenia. Image is a modified version of existing figure

Citations:

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