"Studies on the Role of Gene-Environment Interaction in the pathogenesis of Schizophrenia using a Genetic Animal Model"

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August, 2012

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science in Neuroscience

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Acknowledgments

In the first place, I would like to record my gratitude to Dr. Lalit Srivastava for his supervision and guidance all through my research. He provided me constant encouragement and support in various ways in this project. He is a great scientist and a true teacher who exceptionally inspires and enrich my growth as a researcher and a clinician-scientist want to be.

I gratefully acknowledge Dr. Naguib Mechawar and Dr. Takpan Wong, my committee advisors for their constructive advice and great comments.

I gratefully thank my mentor, Dr. Edith Hamel for her immense support.

Many thanks go to Zhiwen Zeng, a lab mate and a good friend for his helps and support and Dr. Sanjeev Bhardwaj for his technical advice.

I thank, Neda Parinejad for her editing and overall help/comments in preparation of this thesis.

Where would I be without my family? My parents and brother deserve special mention for support. My parents worked on the basis of my learning character, showing me the joy of intellectual pursuit ever since I was a child and I am greatly in debt to both of them.

Finally, I express my appreciation to my best friend and life companion, Houman for all his support.

Abstract

Genetic factors and early adverse environmental events interact to contribute to the pathogenesis of schizophrenia. Here, we investigated if immune-activation during development interacts with a susceptibility gene to produce schizophrenia-related phenotypes. We injected mice with a loss of function mutation in dysbindin-1, a schizophrenia-risk gene and controls with either Polyinosinic:polycytidylic acid (Poly I:C), a viral mimic or saline, at postnatal days (PD)5,6 and 7. At PD60, possible gene-environment (GxE) interaction was studied using tests of behaviours relevant to schizophrenia as well as examination of postnatal neurogenesis. Our data showed a significant effect of genotype on spontaneous locomotion as dysbindin-1 homozygous mice displayed increased locomotor activity. Further, we observed a significant effect of genotype as a decrease in the number of newborn cells in the glomerular layer of the olfactory bulb (OB). Analyses of the data did not reveal an interactive effect between dysbindin-1 and Poly I:C exposure as far as spontaneous locomotion, pre-pulse inhibition of the acoustic startle, novel object recognition memory, elevated plus maze, fear memory, the number of newborn cells in the dentate gyrus and granular cell layer of the olfactory bulb are concerned. These preliminary results demonstrate lack of an interactive effect between this schizophrenia candidate gene and this viral mimic at neonatal periods on selected behavioural and neurobiological measures. Further investigation using different doses of the immune activator and/or different timing of treatment are needed to fully test GxE hypothesis.

RÉSUMÉ

L'interaction entre les gènes et les incidents d'adversités environnementales tôt dans la vie contribuent à la pathogenèse de la schizophrénie. Ici, nous avons étudié si l'activation de système immunitaire au cours du développement interagit avec un gène susceptibe à produire des phénotypes liés à la schizophrénie. Nous avons injecté des souris avec une mutation de perte de fonction dans dysbindin-1, un gène de risque pour la schizophrénie et les contrôles soit avec Poly I: C, une infection virale imiter ou une solution saline, aux jours postnataux (PD) 5,6 et 7. Au PD60, les interactions gène-environnement (GXE) possibles a été étudiée en utilisant des tests de comportements pertinents à la schizophrénie ainsi que l'examen de la neurogenèse postnatale. Nos données ont montré un effet significatif du génotype sur la locomotion spontanée que dysbindin-1 souris homozygotes activité locomotrice affiché augmenté. En outre, nous avons observé un effet significatif du génotype comme une diminution du nombre de cellules nouveau-nés dans la couche glomérulaire de l'OB. Les analyses des données n'a pas révélé un effet interactif entre dysbindin-1 et Poly I: C l'exposition dans la mesure où la locomotion spontanée, pré-impulsion d'inhibition de l'sursaut acoustique, le roman de la mémoire de reconnaissance d'objets, surélevé labyrinthe, la mémoire de peur, le nombre de cellules nouveau-nés dans le gyrus denté et la couche de cellules granulaires du bulbe olfactif sont concernés. Ces résultats préliminaires démontrent l'absence d'un effet interactif entre ce gène candidat schizophrénie et ce virale imiter à la période néonatale sur certains mesures comportementales et neurobiologiques. Une enquête plus approfondie en utilisant des

doses différentes du système immunitaire activateur et / ou différents temps de traitement sont nécessaires pour tester complètement l'hypothèse GxE.

1. INTRODUCTION

1.1 Schizophrenia, an overall view

From turn of the 20th century, when the German psychiatrist, Emil Kraepelin observed a group of his patients develop a deteriorating course of mental illness from early in life, many years have passed. Kraepelin named this condition 'dementia praecox'. The disorder renamed by Eugen Bleuler, schizophrenia, has been widely studied during the years; however, it has still remained one of the great challenges in the field of neuropsychiatry.

Every year, around 20-40 persons per 100,000 are diagnosed with schizophrenia (McGrath, 2006). The life time prevalence of schizophrenia is 1% worldwide (Saha et al., 2005).

Schizophrenia imposes a considerable socioeconomic burden on the patients, their families and caregivers and societies worldwide. Based on the Canada public health agency report (2002), the total direct cost of schizophrenia such as health care, administrative expenditure and loss of productivity is an estimated amount of $2.35 billion and the indirect cost is an additional $2 billion per year (Goeree et al., 1999a, Goeree et al., 1999b, Lopez et al., 2006).

The peak age of onset is late adolescence and early adulthood; however, in females the onset is 5 years later (Abel et al., 2010). The male:female incidence rate is 1.4:1; although

after the age of 40, the incidence in females is higher (Abel et al., 2010). Generally, women respond better to antipsychotic medication and have better prognosis (Abel et al., 2010). Further, women tend to recover better from episodes of psychosis and have lesser frequency and shorter periods of readmission to the hospital and higher frequency of recovery periods (Grossman et al., 2006).There is also evidence that indicate more negative symptoms in male subjects than in females (Ring et al., 1991, Schultz et al., 1997).

The clinical diagnosis of schizophrenia in North America is generally based on the Diagnostic and Statistical Manual, version IV (DSMIV) of the American Psychiatric Association. Presenting symptoms in schizophrenia consist of: 1- positive symptoms (e.g. hallucination, delusion, disorganized speech and behaviour), 2- negative symptoms (e.g. emotional flattening, social dysfunctions). Though not part of the DSMIV diagnostic criteria, significant cognitive impairments in schizophrenia subjects are consistently reported, and are often considered being a "core" problem in the disorder. These cognitive symptoms include, among others, attention deficits, abnormal working memory and executive functions.

The mental disturbance in schizophrenia captures all aspects of brain mental activity from perception and thought to language and behavioural controlling. The presentation is variable across individuals diagnosed with schizophrenia indicating the heterogeneous nature of the disorder. The diverse nature of the clinical presentation of schizophrenia also indicates heterogeneity in the underlying etiological and pathophysiological mechanisms (McGrath, 2008). Many characteristics of schizophrenia overlap with other psychiatric disorders such as bipolar disorder and autism (Berrettini, 2000, Konstantareas

and Hewitt, 2001). Substance abuse, anxiety and depression are the main psychiatric comorbidities observed in schizophrenia(Buckley et al., 2009). The criterion-based approach to schizophrenia does not take into account this heterogeneity and is also atheoritical with regards to the etiology and biology of the disorder. Taking into account the existing pitfalls in the current classifications, it now is debated on whether schizophrenia should be considered a single class of disorder or rather be categorized into groups of distinct disorders namedschizophrenias(Peralta and Cuesta, 2011).

1.2 Neurobiology of schizophrenia

1.2.1 Neurotransmitters

Growing body of evidence supports the involvement of multiple neurotransmitter systems in schizophrenia. Abnormalities in dopamine (DA), Glutamate (Glu), gamma-aminobutyric acid (GABA) and serotonin (5HT) system are all supported by an extensive number of studies. Despite all these reports, the exact neurochemical mechanisms involved in schizophrenia is still unknown. Nevertheless, it is assumed that these multiple neurotransmitter systems interact resulting in the pathophysiology of schizhophrenia (Lang et al., 2007, Stone et al., 2007, Lisman et al., 2008, Dean et al., 2009, Del Arco and Mora, 2009).

Dopamine (DA)

As of yet, the most extensively studied hypothesis in the pathophysiology of schizophrenia is based on abnormal DA transmission. The majority of neurons responsible for DA transmission initiate from the ventral tegmental area (VTA). From VTA, the axons project either to the limbic regions, through the striatum, hippocampus, amygdala and the medial prefrontal cortex (mPFC) and form the mesolimbic pathway or they reach the frontal cortex and shape the mesocortical pathway (Prasad and Pasterkamp, 2009).

The dopamine hypothesis of schizophrenia suggests that an excess of dopaminergic transmission in the limbic system induces psychotic symptoms whereas reduction in dopaminergic transmission in the PFC is the basis for the cognitive deficits and negative symptoms observed in schizophrenia (Davis et al., 1991, Guillin et al., 2007, Schmitt et al., 2009).

The abnormalities in the dopaminergic neural circuitry have been an important focus since it was observed that DA D2 receptor (D2R) blockers alleviate positive symptoms and have antipsychotic effects (Deniker, 1978, Kapur and Mamo, 2003). Furthermore, D2 receptor agonists such as cocaine and amphetamine can aggravate psychosis in schizophrenia patients and even trigger psychotic symptoms in healthy individuals (Garver et al., 1975, Lieberman et al., 1987, Laruelle et al., 1999). Schizophrenia patients have shown amphetamine-induced increase in dopamine release in striatum especially during the peak of the illness (Laruelle et al., 1999). Moreover, neuroimaging studies on schizophrenia patients using positron emission tomography (PET) and Single-photon

emission computed tomography (SPECT) have demonstrated increased D2R occupancy in striatum, indicating an increased D2R occupancy and enhanced dopamine transmission (Zipursky et al., 2005, Remington et al., 2006, Kegeles et al., 2008, Uchida et al., 2008).

Schizophrenia patients have impairments in working memory, a task involving prefrontal cortical dopminergic brain circuitry (Tanaka, 2006, Forbes et al., 2009). Although, the evidence strongly indicates involvement of dopamine transmission in schizophrenia, more investigation is needed to explicate the exact mechanisms involved and the possible interaction with the other neurotransmitters.

Glutamate (Glu)

A number of studies have looked at the dysregulation of excitatory glutamatergic circuitry in the pathophysiology of schizophrenia. These studies indicate hypofunction of N-methyl-D-aspartate (NMDA) subtype of Glu receptors based on the observation that subanesthetic dosages of NMDA antagonists, namely ketamine and phencyclidine (PCP), would induce “schizophrenia like” symptoms in healthy individuals and exacerbate the symptoms in schizophrenia patients (Javitt and Zukin, 1991, Krystal et al., 1994, Olney and Farber, 1995).

Furthermore, decrease in the level of NMDA receptor and

N-Acetylaspartylglutamic acid (NAA), a Glu neuronal marker in different regions of the brain of schizophrenia patients has been reported (Healy and Meador-Woodruff, 2000, Woo et al., 2004, Eastwood and Harrison, 2005). Interestingly, substances that facilitate

the activity of the NMDA receptors such as glycine or D-cycloserine ameliorate cognitive and negative symptoms in schizophrenia patients (Leiderman et al., 1996, Goff et al., 1999).

The glutamate hypothesis of schizophrenia proposes that NMDA receptor hypofunction is the underlying mechanism in schizophrenia.

It is suggested that the abnormal dopamine transmission is due to a hypofunctional Glu system. Projections from the cortical glutamatergic neurons reach the striatum, and control dopaminergic transmission (Laruelle et al., 2003, Stone et al., 2007). Further, an interaction exists between NMDA receptors and dopamine receptors in the PFC and hippocampus (Tseng and O'Donnell, 2004, Sarantis et al., 2009).

Gamma-aminobutyric acid (GABA)

Reports indicate that schizophrenia patients have alterations in GABA neurotransmission. A highly replicated finding is a decrease in Glutamate-decarboxylase-67 (GAD67), the enzyme responsible for GABA synthesis, and GABA membrane transporter-1 in post-mortem brains of schizophrenia patients (Woo et al., 1998, Lewis et al., 1999, Guidotti et al., 2000, Volk et al., 2002, Zhang et al., 2002, Hashimoto et al., 2008, Cruz et al., 2009). Interestingly, the decrease in GABA markers is mainly observed in the chandelier class of parvalbumin-containing interneurons of the PFC in schizophrenia patients and not all interneurons (Lewis et al., 1999, Volk et al., 2002, Hashimoto et al., 2003). It is suggested that the NMDA hypofunction in this subset of interneurons results in reduction

of GABAergic inhibitory function. This is supported by the evidence that NMDA receptor antagonists induce reduction in GAD67 and parvalbumin levels (Coyle, 2004).

The neural network in the brain possesses a synchronized oscillatory activity that is essential for cognitive functions (Uhlhaas et al., 2008). Electrophysiological investigations in schizophrenia patients show abnormalities in neural synchrony and oscillations (Uhlhaas et al., 2008, Doege et al., 2009). GABAergic circuitry in the cognitive substrates (e.g. PFC and Hippocampus) has an important regulatory role in the synchronized oscillations (Lewis and Gonzalez-Burgos, 2008) . This evidence indicates an additional link between the GABAergic circuitry and schizophrenia pathophysiology.

Serotonin (5HT)

The atypical antipsychotic drugs e.g. clozapine, olanzapine and risperidone, have drawn the attention to the involvement of serotonin circuitry in the pathophysiology of schizophrenia. This new generation of antipsychotics shows lesser side effects (Meltzer et al., 2003). They have both antiserotonergic (5HT2 blockade) and antidopaminergic (D2R antagonism) components and increase DA release in the PFC but not so much in the mesolimbic system (Di Pietro and Seamans, 2007, Kuroki et al., 2008). This mechanism may contribute to the prominent ameliorating effect of this group of antipsychotics on negative symptoms (Lublin et al., 2005, Burton, 2006) .

1.2.2 Neuropathology in schizophrenia

Current technologies in neuroimaging have helped in identifying a number of macroscopic abnormalities in brains of schizophrenia patients. Enlarged lateral and third ventricles at the first episode of psychosis in unmedicated patients is a finding reported in a number of studies (Kelsoe et al., 1988, Shenton et al., 2001). This observation at the first stages of the disorder, along with some reports of their non-progressive nature, is in support of the neurodevelopmental theory suggesting alterations in the neurodevelopmental processes as the etiological pathology in schizophrenia (Fannon et al., 2000, Lieberman et al., 2001, Sapara et al., 2007) .

PFC and hippocampus are two main regions associated with schizophrenia abnormalities (Kuperberg et al., 2003, Narayan et al., 2007, Sapara et al., 2007, Nesvag et al., 2008). Structural Magnetic Resonance Imaging(MRI) studies have shown reduction of the cortical thickness in the PFC (Nakamura et al., 2008, Herold et al., 2009) and decreased hippocampal volume in schizophrenia patients (Nelson et al., 1998, Heckers, 2001b).

Both the cortical thinning and increased ventricular volume is also observed in the unaffected siblings of the schizophrenic patients. This suggests a possible link between the morphometric changes in the brain and genetic susceptibility to schizophrenia (McDonald et al., 2006, Goghari et al., 2007, Goldman et al., 2009). Diffusion tensor imaging has indicated a decrease in the density of the white matter regions of the brain such as the corpus callosum (Woodruff et al., 1995, Arnone et al., 2008) .

In terms of microscopic findings, several cytoarchitectural alterations have been observed in the post-mortem brains of schizophrenia patients. A number of studies report the

presence of pyramidal neuron atrophy and decreased neural density in subregions of the brain (Benes and Bird, 1987, Pennington et al., 2008). However, there are also conflicting reports that indicate no change in the number of neurons (Andersen et al., 2004) and even increased neural density (Selemon et al., 2003). Furthermore, a replicated finding in schizophrenia is decreased neuropil size, reduced dendritic arborisation and lower spine density in the prefrontal cortical pyramidal neurons (Glantz and Lewis, 2000, Sweet et al., 2004, Kolluri et al., 2005).

In addition, investigations on post-mortem brains have reported abnormalities in the laminar organization and orientation of the entorhinal and the cingulate cortices (Arnold et al., 1997, Fornito et al., 2009). Abnormalities in migration of a specific subgroup of cells (i.e. NADPH-diaphorase-positive cells) in the frontal and temporal lobe have been observed that further indicates possible alterations in the process of neurodevelopment (Akbarian et al., 1993a, Akbarian et al., 1993b).

1.2 Cognitive deficits in schizophrenia

Cognitive deficits are present long before the onset of full blown schizophrenia, throughout the disorder and even during the controlled phases of the disorder (Niendam et al., 2003, Bowie and Harvey, 2005). The cognitive impairments have great impact on the prognosis of the patients and are considered the core pathology in schizophrenia (Green et al., 2004). Cognitive impairments observed in schizophrenia are also seen in other psychiatric disorders; however these symptoms are more severe in schizophrenia. Schizophrenia patients suffer from impairments in general intelligence, attention,

processing speed, executive functioning, episodic memory, working memory and social interaction (Kalkstein et al., 2010). The current antipsychotic medication is not effective in controlling the cognitive symptoms, leaving the patients with a poor quality of life (Kalkstein et al., 2010, Irani et al., 2011).

Different aspects of neurocognitive deficits in schizophrenia have been studied during the recent years. The PFC and the medial temporal lobe area specially the hippocampus are brain substrates associated with cognitive deficits in schizophrenia (Knable and Weinberger, 1997, Heckers, 2001a, Reichenberg and Harvey, 2007, Minzenberg et al., 2009).

Structural and functional neuroimaging studies indicate abnormalities in the PFC, a pivotal region in cognitive processing (Weinberger et al., 1994, Riehemann et al., 2001, Winterer et al., 2006, Lewis and Gonzalez-Burgos, 2008, McGuire et al., 2008, Voets et al., 2008).

The temporal lobe particularly the hippocampus is also implicated in schizophrenia. Structural imaging studies indicate reduction in the volume of hippocampus, a substrate for declarative memory (Heckers, 2001a). Furthermore, functional imaging studies indicate abnormal activity in the hippocampus during encoding and retrieval of memory (Heckers, 2001a, Hofer et al., 2003, Zhou et al., 2008).

1.4 Neurodevelopmental hypothesis

It is now widely accepted that schizophrenia is a neurodevelopmental disorder. Meaning that it is not a condition that suddenly appears in adulthood; rather, certain alterations occur in the process of neurodevelopment resulting in the abnormalities observed in schizophrenia (Weinberger and Lipska, 1995, Lewis and Levitt, 2002, McGrath et al., 2003, Rapoport et al., 2005).

There are several lines of evidence that support the neurodevelopmental hypothesis of schizophrenia. Cognitive impairments and developmental delays are present at premorbid and prodromal phases of the clinical course of schizophrenia (Fatemi and Folsom, 2009). Congenital anomalies such as agenesis of corpus callosum and stenosis of the sylvian aqueduct have been observed in schizophrenia patients (Lloyd et al., 2008, Fatemi and Folsom, 2009).

Presences of neurologic soft signs in children who are later diagnosed with schizophrenia have also been reported (Barkus et al., 2006). Further, deficits in attention and psychomotor performance, social impairment, mood abnormalities and intense anxiety have been seen to occur more commonly in children considered high-risk due to having a schizophrenic parent (Fish, 1957, O'Neal and Robins, 1958, Fish et al., 1992, Fatemi and Folsom, 2009). Furthermore, physical aberrations such as low-set ears, facial asymmetries, epicanthal eye folds, abnormal skull measurements and wide spaces between the first and second toes in children who later develop schizophrenia are also suggestive of developmental anomalies that occur during prenatal period (Gualtieri et al., 1982, Ismail et al., 1998, Waddington et al., 1999).

These impairments are indicative of a neurodevelopmental basis for the disorder. A number of these evidence signify deficits due to alterations in the normal development of the temporo-limbic neural circuits in the brain during pre- and postnatal development (Weinberger and Lipska, 1995, McGrath et al., 2003, Rapoport et al., 2005, Fatemi and Folsom, 2009). Several post-mortem studies on schizophrenia subjects have shown cytoarchitectural changes in the brain suggestive of a disruption in the course of neurodevelopment (Arnold et al., 1991, Akbarian et al., 1993b, Kirkpatrick et al., 1999). Among these pathological findings are disturbances in neurogenesis processes (e.g. neuronal migration) and cortical lamination in the temporal lobe structures (Kovelman and Scheibel, 1984, Jakob and Beckmann, 1989, Arnold et al., 1991, Akbarian et al., 1993a). Further, presence of non-progressive enlargement of lateral and third ventricles, decrease in grey matter thickness and lack of neurodegenerative processes such as astrogliosis (Roberts and Crow, 1987, Arnold, 1999, Falkai et al., 1999) support brain maldevelopment as the underlying pathogenic mechanism in schizophrenia (Pfefferbaum and Zipursky, 1991, Lewis and Levitt, 2002, Vita et al., 2006, Pagsberg et al., 2007, Fatemi and Folsom, 2009). Post-mortem studies have been further followed by animal studies. By means of using different methods such as X-ray irradiation and an antimitotic drug, methylazoxymethanol acetate (MAM), disruption in neurodevelopmental processes such as neurogenesis (e.g. neural maturation) and synaptogenesis have been induced resulting in morphological and behavioural changes associated with schizophrenia (Gelowitz et al., 2002, Moore et al., 2006).

Another promising schizophrenia model based on the neurodevelopmental hypothesis of schizophrenia is the neonatal ventral hippocampal lesion (NVHL) model. The ventral

hippocampus sends glutamatergic projections to the PFC (Carr and Sesack, 1996). The period of postnatal days 7 to 9(PD7-9) is critical for the development of the hippocampal formation in rats (Minkwitz, 1976). This point coincides with the peak in the dendritic and axonal growth and thus the hippocampus is susceptible to adversities (Minkwitz, 1976, Chen and Strickland, 1997, Sapolsky, 2002). Lipska et al. demonstrated that bilateral excitotoxic lesions in rat hippocampus at PD7 results in occurrence of schizophrenia-like behaviours in adulthood (Lipska et al., 1993). These rats show hyperlocomotion in response to stress, psychostimulants and DA agonists (Lipska et al., 1993, Flores et al., 1996, Wan and Corbett, 1997, Conroy et al., 2007, Berg and Chambers, 2008). They also display deficits in sensorimotor gating (Lipska et al., 1995, Grecksch et al., 1999), social interactions (Becker et al., 1999, Vazquez-Roque et al., 2012), working memory (Gruber et al., 2010) and conditioned emotional response (Angst et al., 2007). Further, a number of typical and atypical neuroleptics have shown to control some of the behavioural abnormalities in this model (Lipska and Weinberger, 1994, Le Pen and Moreau, 2002).

1.5 Etiology

The etiology of schizophrenia is heterogeneous; genetic factors and early adverse environmental insults contribute to the pathogenesis of schizophrenia. Until recently, researchers tended to study schizophrenia either from genetic or environmental perspective alone (Oliver, 2011). Now, however, scientists are considering that genetic and environmental factors may interact leading to the occurrence of schizophrenia.

1.5.1 Genetic factors

Schizophrenia is a complex disorder with high heritability (Robertson et al., 2006).

Genetic factors contribute significantly to the etiology of schizophrenia (Kendler et al., 2011, Riley and Kendler, 2011, Mulle, 2012) . This is supported by family, twin and adoption studies that indicate high schizophrenia prevalence in the relatives of schizophrenia individuals (i.e. more than 50% chance in monozygotic twins, 16% in dizygotic twins and less than 10% in first degree relatives)(Lewis and Levitt, 2002, Chen et al., 2009, Li et al., 2009). In studies on schizophrenic subjects who were adoptees, it was observed that the incidence of schizophrenia and impairments in executive functioning was significantly higher in biological parents than in the adoptive parents (Kety et al., 1994, Wahlberg et al., 1997). Although strong evidence supports the major role of genetic factors in the pathogenesis of schizophrenia, the mode of transmission is yet unclear and evidently does not follow Mendelian inheritance (Tsuang et al., 1991, Owen et al., 2005). Genetic association and linkage studies have identified a number of chromosomal loci that increase risk to schizophrenia. These studies provide new evidence on the association of variations in several genes e.g. dysbindin-1, Neuregulin-1, DISC-1, COMT and GAD-1 with schizophrenia (Blackwood et al., 2001, Shifman et al., 2002, Straub et al., 2002, Fallin et al., 2003, Stefansson et al., 2003, Maziade et al., 2005, Suarez et al., 2006, Escamilla et al., 2007, Allen et al., 2008) . The function of these genes has individually been studied through genetic animal models (Chen et al., 2006, Arguello et al., 2010). Interestingly, these schizophrenia susceptibility genes are

involved in different stages of the neurodevelopmental processes and functions (i.e. cell proliferation, migration, axonal outgrowth, synaptogenesis, synaptic neurotransmitter levels) and further support the neurodevelopmental hypothesis of schizophrenia (Chen et al., 2006).

Disrupted-in-schizophrenia-1 (DISC-1) was identified in a Scottish family with multiple cases of schizophrenia, bipolar disorder and major depression clustered in the pedigree (Millar et al., 2001). DISC-1 is highly expressed during the development of the neural system and neural trajectories and it plays an important role in neurogenesis, neural migration, neuronal outgrowth and synaptogenesis (Hattori et al., 2007, Niwa et al., 2010).

Cathechol-O-Methyl transferase (COMT) catalyzes degradation of catecholamines and has been linked to cognitive deficits in schizophrenia (Egan et al., 2001, Eisenberg et al., 2010) . Val containing allele of COMT has an accelerating role in catabolism of catecholamines (e.g. DA catabolism in PFC) and this has been associated with impairments of frontal lobe dependent cognitive functions (Tunbridge et al., 2006, Babovic et al., 2007).

Neuregulin-1 is another schizophrenia candidate gene under the investigation (Williams et al., 2003). Basically, neuregulins bind to Erb B receptor tyrosin kinase (Williams et al., 2003, Tosato et al., 2005). This gene has a pivotal role in the regulation of glial cells, neural migration and expression of neurotransmitter receptors such as GABA-R and NMDA-R (Tosato et al., 2005, Sei et al., 2007, Pitcher et al., 2011).

It should be noted that the risk genes have small effect sizes themselves and do not explain all clinical phenotypes. This could partly be due to the possibility of gene-gene interaction, where each genetic mutation captures certain aspects of schizophrenia called 'endophenotype' rather than the full spectrum of the disorder.

Furthermore, it is believed that genetic variations, by altering common aspects of neurodevelopment or plasticity, render the brain more susceptible to environmental risk factors leading to "full-blown" disorder and divergent clinical phenotypes (Lewis and Levitt, 2002).

Dysbindin-1

Among the candidate genes associated with schizophrenia, DTNBP-1, located on the locus 6p22.3 is one of the most promising genes and studied in Dr. Srivastava's lab for several years (Straub et al., 2002, McGuffin et al., 2003, Bhardwaj et al., 2009). Several studies show an association between a single nucleotide polymorphism (SNP) or a number of SNPs (haplotypes) in this gene with schizophrenia (Straub et al., 2002, Schwab et al., 2003, Bray et al., 2005). Post-mortem studies on schizophrenia individuals show dysbindin-1 reduction in the two main regions affected in schizophrenia, the prefrontal cortex (PFC) and hippocampus (Talbot et al., 2004, Tang et al., 2009). The reduction in the level of dysbindin-1 in schizophrenia brain makes an animal model with lack of function mutation in this gene, a worthy resource for studying the role of dysbindin-1(Talbot, 2009). Most of the understanding on this gene is from mice with a natural mutation in dysbindin-1 gene, named Sandy that demonstrate many behavioural

and pathophysiological deficits associated with schizophrenia (Talbot, 2009). A spontaneous 38.129 Kb deletion in DTNBP-1 gene emerged in DBA/2J background that resulted in a 58 amino acid loss in coiled-coil domain of dysbindin-1 and a non-functional protein (Talbot, 2009). The term sandy is used for the homozygote dysbindin-1 muted mice and refers to the sandy coat color which is due to the lack of melanosome formation (Bhardwaj et al., 2009, Talbot, 2009). The name dysbindin or Dystrobrevin binding protein is due to its discovery as a binding partner to Dystrobrevin protein involved in muscular cell skeletal assemblies (Benson et al., 2001). Further, dysbindin-1 is a part of biogenesis of lysosome-related organelles complex (BLOC-1)(Iizuka et al., 2007).This complex is involved in protein trafficking to lysosome-related organelles (LRO) (e.g. melanosomes, platelet granules and synaptic vesicles) which is both crucial in LRO maturation and also function of the proteins delivered (e.g. D2 receptors and its lysosomal degradation) (Iizuka et al., 2007, Talbot, 2009). Dysbindin-1 protein is expressed both pre- and postsynaptically throughout the brain and it's isoforms A, B (does not exist in mice) and C are associated with post-synaptic density (PSD) and synaptic vesicles (Talbot et al., 2011).

Shao et al. have studied the consequence of reduction in dysbindin-1 expression in drosophila. They found that reduced levels of presynaptic dysbindin-1 lead to a reduction in glutamate transmission. On the other hand, decreased expression of dysbindin-1 in glial cells causes hyperdopaminergic activities. (Shao et al., 2011).

As previously mentioned, sandy mice demonstrate several schizophrenia-related cognitive and pathophysiological deficits (Bhardwaj et al., 2009, Cox et al., 2009). These mutant mice demonstrate attenuated locomotor activity in response to DA agonists in

single amphetamine administration but increased locomotor activity in multiple injections (Bhardwaj et al., 2009). The data from this lab (unpublished) and other studies show that pre-pulse inhibition (PPI) of the acoustic startle is not different from the wild type. However, Papaleo at.al have reported that dysbindin-1 mutants on C57BL/6 background have increased acoustic startle and prepulse inhibition(Papaleo et al., 2012). Further, sandy mice have not shown anxiety-related behaviours (Cox et al., 2009). They have shown impairments in social behaviour and learning and memory as evidenced by object recognition test (on DBA/2J background), fear memory (on DBA/2J background) and spatial learning and memory (on C57BL/6 background) (Bhardwaj et al., 2009, Cox et al., 2009). Studies have demonstrated discrepant results on locomotion in open field but decreased locomotion is observed in one study in C57BL/6 background (Talbot, 2009). Further, sandy mice have shown increased dopamine release in the limbic system, cell surface D2 receptor over-expression in PFC, decreased Glu release in PFC and hippocampus, decreased mEPSC and eEPSC in PFC pyramidal neurons, decreased excitability of parvalbumin positive interneurons due to potential NMDA-receptor hypofunction (Talbot, 2009, Papaleo et al., 2010, Papaleo and Weinberger, 2011).

In a study, Tognin et al. investigated the effects of high-risk allele (AA) of dysbindin-1 gene compared with the low-risk allele (TT) on the white and gray matter thickness in regions of the brain implicate in schizophrenia. Their results demonstrated that individuals with the high risk allele had reduced gray matter volume in the left anterior cingulate gyrus and decreased white matter volume in the left medial frontal area. The study was performed on children between 10-12 years of age and the results support a

role of dysbindin-1 neurodevelopmental processes implicated in schizophrenia (Tognin et al., 2011).

1.5.2 Environmental Risk factors

The estimated 60-80% heritability of the disorder clearly points out that although the etiology of schizophrenia is strongly determined by genetic factors, it also points out the non-genetic component of its pathogenesis (McGuffin et al., 1984, Onstad et al., 1991, Brown, 2011). This is further supported by the retrospective epidemiologic studies that have shown the association of environmental factors and increased schizophrenia risk (Bayer et al., 1999, Howes et al., 2004). These factors may both predispose the susceptible patients and also later play a role as the triggering factor (e.g. stress) just before the full-blown onset of the disorder. Notable environmental risk factors for schizophrenia include maternal/postnatal infections, obstetric complications, maternal stress, neonatal seizure, paternal age, urbanicity, migration and adolescence drug abuse (McDonald and Murray, 2000, Bresnahan et al., 2005, Opler and Susser, 2005, Brown, 2011).

Multiple population-based studies have demonstrated that people who reside in urban areas are at greater risk of schizophrenia (Marcelis et al., 1999, Mortensen et al., 1999, March et al., 2008). This increase might be due to specific environmental factors and characteristics of urban regions such as microbial pathogens, toxins, diets and sociocultural factors (Brown, 2011).

A quite replicated finding is the association of schizophrenia occurrence with births in late winter and early spring. The estimated increase in risk is 5-15% (Bradbury and Miller, 1985, Torrey et al., 1997, Davies et al., 2003) and it is possible that this is due to higher incidence of infections during these seasons (Brown, 2011).

A number of epidemiological studies show that early immune-activation by bacterial and viral agents are a risk factor for the occurrence of schizophrenia. The first study of its kind was the study of Mednick et al. on the Finish population who were foetuses during the 1957 influenza epidemic. The study showed higher risk of admission to hospital with diagnosis of schizophrenia in these individuals (Mednick et al., 1988). Studies on the role of early infection were replicated many times e.g. in Japan (Kunugi et al., 1995) and Britain (Kendell and Kemp, 1989, O'Callaghan et al., 1991). Brown et al. (Brown et al., 2004) studied a cohort of individuals born during 8 years from 1959 to 1967 in California. The individuals were under a health plan that required mother's sera to be drawn during pregnancy and stored. In the study, they assessed the sera for influenza antibody and they also did a follow up of the individuals born to these mothers for later diagnosis of schizophrenia spectrum disorders (SSD). The results indicate a 7-fold higher rate of SSD occurrence in individuals whose mothers were affected by influenza during their pregnancy. These results and similar studies indicate a role of early (perinatal and even adolescence) exposure to environmental factors in schizophrenia occurrence (Degenhardt and Hall, 2006, Brown, 2011).

In a study by Koponen et al., the association between history of childhood CNS infection and schizophrenia and other psychosis was assessed. This study on a birth cohort in northern Finland found a positive association between postnatal viral CNS infections and

schizophrenia. The estimated odds ratio for schizophrenia after a viral CNS infection was 4.8 (Koponen et al., 2004).

A number of animal models have been used to test the maternal infection and immune activation hypothesis (Boksa, 2010, Meyer and Feldon, 2010).Studies have used immune-activators such as the influenza virus, Lipopolysaccharide (LPS) or Polyinosinic:polycytidylic acid (Poly I:C) to model early infections (Meyer et al., 2005, Boksa, 2010, Meyer and Feldon, 2010).

These studies, administering the above-mentioned immune activators to pregnant rodents, demonstrate cognitive and behavioral changes in the adult offsprings, e.g., decreased prepulse inhibition (PPI) of acoustic startle, increased sensitivity to DA agonists, impaired spatial and object recognition memory and social interaction deficits (Boksa, 2010).

LPS is a bacterial mimic and an endotoxin obtained from the cell wall of gram negative bacteria (Miller et al., 2005a). LPS binds to Toll-like receptors 2 and 4 (TLR-2 and TLR-4) and triggers the downstream signaling cascade that leads to the production of proinflamatory cytokines such as tumor necrosis factor-α (TNF- α) and interleukin-6(IL-6)(Miller et al., 2005b). Prenatal and early postnatal (Walker et al., 2004, Boksa, 2010, Walker et al., 2012) administration(subcutaneously or intraperitoneally) of LPS to rodents has led to abnormal behaviors in adult animals (Fortier et al., 2004, Meyer et al., 2005, Romero et al., 2007, Boksa, 2010). In close relation to the time and dosage of LPS administration, rodents have demonstrated changes such as increased locomotor activity, deficits in pre-pulse inhibition (PPI) of acoustic startle and impaired novel object

recognition memory (Boksa, 2004, 2010). Walker et al. have shown that neonatal administration of LPS results in anxiety-like behaviours in adult rats (Walker et al., 2004).

Inducing influenza infection in pregnant rodents has been shown to result in behavioral and neuropathological abnormalities associated with schizophrenia and autism in adult offspring (Fatemi et al., 1998, Fatemi et al., 2002, Shi et al., 2003, Fatemi, 2009).

Furthermore, based on the notion that cytokines mediate the adverse effects of bacterial and viral infections, rodent models of proinflamatory cytokines have been studied (Meyer et al., 2005). In this regard, prenatal administration of IL-6 has been investigated and has shown behavioral, structural and pathophysiological changes relevant to schizophrenia (Samuelsson et al., 2006, Smith et al., 2007).

Models for testing Infection hypothesis: Poly I:C

Poly I:C (Polyinosinic:polycytidylic acid) is a synthetic double-stranded RNA and a viral mimic. It stimulates the release of pro- and anti- inflammatory cytokine (TNF-α, IL-1β and IL-10) through binding to Toll-like receptor3 (TLR-3) present on the endosomal surface (Gilmore et al., 2005, Meyer et al., 2006, Matsumoto and Seya, 2008). Poly I:C administered gestationally, induces behavioural and pathophysiological alterations associated with schizophrenia, namely deficits in prepulse inhibition (PPI) of the acoustic startle, decreased exploration in open field, deficits in social interaction, deficits in working memory and alterations in spatial learning and memory in Morris water maze (MWM)(Meyer et al., 2005, Boksa, 2010). Apart from maternal immune-activation

models, Ibi et al. have shown that neonatal injection of Poly I:C results in impaired novel object memory, PPI deficits, impairment in social interaction and increased anxiety-like behaviours in open field (Ibi et al., 2009).

It is shown that glutamatergic and dopaminergic systems are affected in offspring of mothers injected with Poly I:C. This is demonstrated in the study showing decrease in NMDA receptors in the hippocampus of offspring of gestationally challenged rodents (Meyer et al., 2008). Also, administration of MK-801, an NMDA receptor antagonist has shown to have an enhanced effect on locomotion (Zuckerman et al., 2003, Zuckerman and Weiner, 2005). Among the evidence supporting the dopaminergic involvement in maternal Poly I:C models are amphetamine-induced locomotion, impairment in latent inhibition and deficits in working memory (Ozawa et al., 2006). Following amphetamine administration, an increase in the dopamine release in striatum was observed in the offspring (Zuckerman and Weiner, 2005). Further, an increase in tyrosine hydroxylase in striatum and a decrease in D1 and D2 receptor in the prefrontal cortex is reported in prenatal Poly I:C model (Meyer et al., 2008, Meyer and Feldon, 2009). Moreover, a decrease in reelin and parvalbumin positive cells in the prefrontal cortex is observed in the same model (Meyer et al., 2008).

Miranda et al. have reported that gestational administration of Poly I:C prevents the replication of embryonic neuronal stem cell and inhibits formation of superficial layers of the neocortex (De Miranda et al., 2010). Another group has also investigated the effects of maternal challenge with Poly I:C on cortical development. They have too reported disruption in the superficial layers of the neocortex and alterations in synaptogenesis (Soumiya et al., 2011).

1.5.3 Gene-environment interaction

Taking into account the evidence supporting the role of genes and environmental insults in occurrence of schizophrenia-related abnormalities, a hypothesis is built around an interplay of both as the key feature in the etiology of schizophrenia(Oliver, 2011) .

The importance of gene-environment interaction in schizophrenia is underscored by the fact that although monozygotic twins share 100% of the genes, the concordance for schizophrenia is only 50% (Chen et al., 2009, Li et al., 2009). Indeed, many genetic epidemiological studies now support the role of an interaction between genetic susceptibility and pre- and perinatal environmental insults in occurrence of schizophrenia. Human epidemiologic studies using positive family history as a proxy for genetic susceptibility and different environmental factors such as early infection have studied this interplay (Oliver, 2011).

Among the studies of gene-environment interaction in schizophrenia is the Dunedin study that looked at the interactive effect of catechol-O-methyltransferase (COMT) *val/val* genotype and early high dose cannabis abuse in increasing the risk of schizophrenia (Caspi et al., 2005). COMT *val/val* genotype is associated with impairments in the prefrontal cortical functions (Henquet et al., 2005). Individuals with this polymorphism have shown higher sensitivity to effects of the active component of cannabis in producing psychosis and cognitive impairments (Henquet et al., 2006).

A study by Nicodemus *et al.* looked at the effects of serious obstetric complications in individuals carrying single nucleotide polymorphisms (SNPs) in genes regulated by hypoxia/ischemia or those involved in vascular function such as AKT-1, BDNF and DTNBP-1. This investigation showed higher risk of schizophrenia as a result of the interaction (Nicodemus et al., 2008).

Clarke et al. (Clarke et al., 2009) studied around 9000 individuals born in Finland from 1947 to 1990 whose mothers had been hospitalised for pyelonephritis (upper urinary tract infection). The control group in this study were their siblings who were not exposed to the infection as foetuses. The results showed the risk percentage in no infection/no family history group to be 0.23%, infection only group 0.32%, family history positive only group 0.58% and family history positive/infection group 1.09%. The analysis also showed that 38-46% of individuals with schizophrenia developed the disorder as a result of gene-environment synergism.

Accordingly, schizophrenia is viewed as a polygenic/multifactorial disease with multiple genetic polymorphisms interacting with multiple environmental factors throughout development (Lewis and Levitt, 2002, Oliver, 2011). Bearing in mind the limitations in interpretation of results from animal studies, certain behavioural correlates of schizophrenia are used in animal studies (Wilson and Terry, 2010). Among them are histopathological (e.g. decrease in neurogenesis processes, neuropils, neuronal density), neuroanatomical (e.g. ventricular enlargement, decreased frontal and temporal grey matter volume) and behavioural (e.g. deficits in attention, learning and memory and sensorimotor gating) characteristics of schizophrenia (Wilson and Terry, 2010).

An animal study on the influence of environmental risk factors in the context of genetic susceptibility to schizophrenia is conducted by Oliver et al..This group looked at the effects of prenatal stress in mice carrying mutations in synaptosomal-associated protein of 25 kDa (SNAP-25) and observed impairments in social interaction and an enhancement in sensorimotor gating deficits as a result of the interaction; furthermore, the latter was reversed with antipsychotics (Oliver and Davies, 2009).

Abazyan and colleagues have looked at the interaction between mutant human disrupted-in-schizophrenia 1 (mhDISC1) and maternal immune challenge with Poly I:C in mice. They showed that the interaction results in increased anxiety and decrease in density of spines on dendrites of granule cells of the hippocampus (Abazyan et al., 2010).

The interaction between DISC-1 and neonatal Poly I:C administration was also assessed. It was reported that the interaction results in deficits in object recognition memory and fear memory, while PolyI:C treatment by itself had lesser effect on wild-type mice. Furthermore, PolyI:C-treated DISC1 mutant mice demonstrated alterations in social interaction. Interestingly, additive effects of PolyI:C and DISC1 mutants lead to a reduction in the number of parvalbumin-positive interneurons in the medial prefrontal cortex(Ibi et al., 2010).

A more recent study looked at the interaction between prenatal immune activation by Poly I:C and mutation in Nurr-1, a transcriptional factor essential for development of the dopaminergic system. They demonstrated that the interactive effect between the named gene and environmental factor results both in additive and de novo phenotypes. The additive effects were observed in hyperlocomotion and sensorimotor gating deficit and

the de novo phenotype was alterations attentional shifting and sustained attention. Furthermore, they showed that this interaction produces developmental abnormalities in the ventral striatal and prefrontal cortical dopaminergic system (Vuillermot et al., 2012).

2. HYPOTHESIS AND RESEARCH PLAN

The epidemiological studies deduce interaction using imprecise measures of environment and genetic variables on broad clinical phenotype (Oliver, 2011). Considering the limitations in human studies, animal models are a great mean for more precise study and featuring of schizophrenia related abnormalities(van Os et al., 2008, Oliver, 2011). Thus, we need hypothesis-driven animal studies where genetic and environmental variations are controlled to better identify the nature of interaction as well as plausible biological pathways through which synergism between gene and environment is realized.

The working hypothesis, I developed for my thesis project is that a negative environmental event, viz. neonatal immune activation and a schizophrenia risk gene, viz., dysbindin-1 will interact to result in altered neurodevelopment and schizophrenia-related behaviour in mice.A pivotal study relevant to our hypothesis is a genetic investigation in human subjects indicating that dysbindin-1 gene interacts with IL3 gene to increase the risk of schizophrenia (Edwards et al., 2008). IL3 is a cytokine secreted by the immune cells. Furthermore, schizophrenia patients have shown imbalance in the immune system

(Avgustin et al., 2005). Therefore, assessment of the interactive effect of dysbindin-1 and immune challenge would be plausible.

Strong yet partial capturing of schizophrenia-related phenotypes by dysbindin-1 mutant and Poly I:C immune activation models leads to the specific hypothesis that:

1) Early neonatal immune activation with Poly I:C in dysbindin-1 mutant mice will synergistically enhance behavioural deficits or result in emergence of schizophrenia relevant behavioural phenotype not seen in dysbindin-1 mutation or immune-activation alone .

2) As a direct result of the interaction, early postnatal neurogenesis will be reduced in the hippocampus and olfactory bulb of dysbindin-1 mutant mice brain. In this study, we have exposed mice to neonatal immune-activation. It must be noted, the basis for choosing neonatal period for injection of Poly I:C in this study is the notion that this period in rodents corresponds to the second trimester of human gestation, based on statistical comparison between species such as mice with humans in terms of neurodevelopmental events (http://translatingtime.net/). In addition, the process of neurodevelopment continues after birth and is a period where the neural system is still very susceptible to environmental insults. Also, studies of neonatal exposure to environmental insults have shown abnormalities associated with schizophrenia in adulthood (Rothschild et al., 1999, Koponen et al., 2004, Jenkins et al., 2009). Further, in maternal infection the effect of the infective agent on neurodevelopment is only indirect and through cytokines whereas in neonatal injection the direct effects could also be studied.

3. MATERIALS AND METHODS

3.1 Animals

The mice on C57BL/6 background, all from heterozygote-heterozygote mating were housed and bred in our animal facility. The temperature was maintained at 21±1 °C on a 12/12 h light/day schedule (lights on 08:00–20:00). All animals had free access to standard mouse chow and tap water. Due to limited number of animals, we did not assess maternal behaviours toward the pups; thus, we cannot rule out the possibility that maternal care differences exist with respect to genotype and treatment.

Age-matched mice from the three genotypes were group housed. Maximum 5 mouse per cage. All animals used in the experiments were healthy and active, and all experiments were conducted in accordance with the guidelines of the Canadian Council for Animal Care, and approved by the McGill University Animal Care Committee.

3.2 Genotyping

All mice were genotyped using a duplex polymerase chain reaction (PCR). The primers for the wild-type gene, producing a PCR product of 472 base pairs, were SE3R (5'-AGCTCCACCTGCTGAACATT-3') and SE3F (5'-TGAGCCATTAGGAGATAAGAGCA-3'). The primers for the dysbindin-1 mutant gene, producing a product of 274 base pairs, were sandy forward (SF) (5'-TCCTTGCTTCGTTCTCTGCT-3') and sandy reverse (SR) (5'-CTTGCCAGCCTTCGTATTGT-3'). The 472-base-pair product is detected only in wild type and Sdy/+ mice, while the 274-base-pair product is detected only in the Sdy/+ and Sdy/Sdy mice.

For a master mix of 25 µL, 2.5 µL of Buffer 10X (200 mM Tris-HCl (pH 8.4), 500 mM KCl) (provided with the Taq DNA polymerase enzyme; Invitrogen) was used. The volume and concentration of primers (custom made, Invitrogen) was 0.25 µL and 100 µM, respectively. The volume and concentration for DNTP stock (Invitrogen) was 10 µ M and 0.5 µL and for MgCl2 (provided with the Taq DNA polymerase; Invitrogen) 50 mM and 1 µL. The concentration of Taq DNA polymerase (Invitrogen) was 5 U/µL and the volume used was 0.2 µL.

The setting of the thermocycler (Veriti™ Dx; Applied biosystem), for 35 cycles of PCR amplification were as follows:

Denature 95°C for 20 seconds

Anneal 55°C for 20 seconds

Extend 72°C for 30 seconds

The reaction was maintained at 4°C after cycling. The samples were loaded on 2% agarose gel prepared in TBE. 2 µL of ethidium bromide was added to the agarose solution during preparation. The 274 and 472 base pair sequences were separated after 20 minutes at the voltage of 140 mV.

DNA extraction: DNA was extracted from 1-2 mm of tail snips using Hot Shot genomic DNA preparation. The Alkaline lysis reagent used consisted of sodium hydroxide, 25mM and EDTA, 0.2 mM. 75 µL of this reagent was added to the tails in 0.2 ml tubes and heated at 95 °C for 30 minutes, cooled to 4°C and mixed with 75 µL of Tris-HCL, 40 mM as the neutralization buffer.

3.3 Animals and Treatment

Two cohorts of animals, one for the behavioural tests (n=7-10/group) and the other for the neurogenesis (n=3-4/group) were used in this study.

Sandy homozygote(Sdy/Sdy), heterozygote (Sdy/+) and wild-type (WT) mice, bred on C57BL/6 background, all from heterozygote-heterozygote breeding were marked and taken sample of for genotyping by cutting the paw fingers at P4. Neonates of each genotype (Sdy/Sdy, Sdy/+, and WT) in each litter were divided into two groups with one receiving Poly I:C and the other saline. The neonates were injected with either Poly I:C (4 mg/kg ,1mg/ml, high molecular weight Poly I:C reconstituted in sterile normal saline based on manufacturer's instructions; Invivogen) or an equal volume of normal saline at postnatal day (PD) 5,6 and 7. The injections were done intraperitoneally using 10 µL Hamilton syringes (26" gauge needle), between 11 am to 1 pm. Using this paradigm, we generated six groups of animals: WT saline, WT Poly IC, (Sdy/+)- saline, (Sdy/+)-Poly I:C, (Sdy/Sdy) -saline and (Sdy/Sdy)- Poly I:C.

3.4 Behavioural Assessments

Behavioural tests started when mice reached the age of 2 months. The sequence of tests conducted starting from the least to the most stressful as follows: Spontaneous locomotion test followed next day by the assessment of novel object recognition memory. After a 4-day interval, Pre-pulse inhibition of the acoustic startle was conducted. Two

weeks later, the anxiety was tested using elevated plus maze. Three weeks after elevated plus maze, fear memory was tested.

3.4.1 Spontaneous Locomotor activity and stereotypy

Locomotor activity is a component of exploration in rodents and the mesolimbic dopamine level effects locomotion. Many pharmacological and genetic factors in rodents effect locomotion. At the same time, behaviours are affected by locomotor activity including learning and memory and anxiety state. In schizophrenia, sensitivity exists towards dopamine agonists, inducing hyper-locomotion (Bhardwaj et al., 2009). In this project, although the animals are not pharmacologically challenged with a dopamine agonist, the baseline spontaneous locomotion would be an essential indicative of the dopamine level.

Method: Locomotor activity measurements were conducted in 12 activity chambers (L×W×H=17.5 cm ×10 cm ×26cm) housed in a dimly lit room between 9:00 am to 4:00 pm. Each chamber was equipped with two photoelectric switches; light beam interruptions from each chamber were monitored and analyzed by software (ACTANAL, Concordia University, Montreal, QC). The animals were placed in individual testing chambers and their spontaneous locomotor activity was recorded. Measurement of locomotion (horizontal movement), exploration (sniffing and rearing) and stereotypy (movements without displacement) was done while mice were located in the activity box for 90 minutes. Total distance traveled was measured for each animal and analysed using two-way ANOVA.

3.4.2 Novel object recognition memory

Novel object recognition memory test is based on the premise that rodents explore novel objects more than familiar objects if they remember the familiar one (Papaleo et al., 2011). This test uses the natural tendency of animals to explore the environment without being reinforced by positive rewards (Chen et al., 2006). Novel object recognition is linked to the functions of hippocampus and parahippocampal area such as the perirhinal cortex; areas that are implicated in schizophrenia pathology (Chen et al., 2006). Among the cognitive domains impaired in schizophrenia are working, declarative and visual learning and memory (Redrobe et al., 2010). Novel object recognition test is recommended by the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) to test visual learning and declarative memory (Redrobe et al., 2010).

Method: A chamber (L×W×H: 45×45×45 cm) made of dark Plexiglas was placed in a quiet room. Mice acclimation to environment (testing bin) for 3 days and duration of 20 minutes, was followed by 5 minutes exploration of two identical new objects (toys with various shapes, Dollar Store) at day 4 (familiarization). After 5 minutes of delay (retention) animals were exposed to one identical and one novel object for 3 minutes. The activity was monitored by videotape and analysed by observers blind to the experimental condition. Exploratory activity was measured when the head of the animal was oriented 2-3 cm from the object and towards it or at least one forepaw was on the object or the mouse was sniffing or licking the object. The ratio of novel object exploration time over total exploration time for both objects was assessed at 0.5 chance level using one-sample

t-test. The ratio for each group were also compared using two-way ANOVA ($P<0.05$ considered significant).

3.4.3 Pre-pulse inhibition of acoustic startle (PPI)

A test for sensorimotor gating in rodents as well as humans is based on the decrease in startle response to a loud acoustic stimulus when it is preceded by a weaker acoustic stimulus. PPI is observed among many species including humans and rodents and studies show that dopamine which has an important role in schizophrenia, regulates sensorimotor gating Alteration in PPI is a consistent finding in schizophrenia patients and their unaffected relatives (Swerdlow et al., 1994, Kumari et al., 2005a, Kumari et al., 2005b). In line with the dopamine hypothesis of schizophrenia and presence of PPI deficits in schizophrenia patients, we chose to assess sensorimotor gating deficits in our study.

PPI-response to psychostimulants and amelioration with antipsychotics would have been interesting to assess; however, we aimed to primarily look at different behaviours on the animals and any treatment in the course of the experiments would have unabled us to further assess other behaviours. Therefore, this remains an important aspect to assess in our future plan.

Method: SR-LAB system (San Diego Instruments, San Diego, CA, USA) comprising two sound-attenuating chambers, each equipped with a cylindrical Plexiglas animal enclosure (length 16 cm, inner diameter 8.2 cm) were used. A speaker was positioned 24 cm directly above the enclosure provided the broadband tone pulses. A piezoelectric accelerometer affixed to the animal enclosure frame was used to detect and transduce

motion resulting from the animals' startle response. Tone pulse parameters were controlled by a microcomputer using the software package (SR-LAB) and interface assembly that also digitized, rectified, and recorded stabilimeter readings.

In this experiment, a background noise of 70 dB, prepulse intensities of 76 dB, 79 dB, 82 dB, 85 dB for 30 ms and a burst stimulus of 120 dB were used. The startle response is measured in 32 trials. Mean of acoustic startle response amplitude was measured. The mean startle response was analysed using two-way ANOVA, and if indicated, by post-hoc test considering $P<0.05$ as significant.

3.4.4 Elevated plus maze

Elevated plus maze is a widely used test for assessment of anxiety. This rather simple task consists of an elevated maze with four arms (two open and two enclosed) that form a plus. The elevated plus maze is based on the notion that rodents' have tendency toward dark, enclosed spaces (approach) and have an unconditioned fear of heights/open spaces (avoidance) (Rodgers and Dalvi, 1997, Walf and Frye, 2007). The assessment of anxiety behaviour of rodents is calculated by using the ratio of time spent on the open arms to the time spent on the closed arms.

Method: Behaviour was recorded over 5 min in an elevated plus maze 70 cm above the ground, consisting of two closed and two open arms, each 50 cm×5 cm in size. The test instrument was built from grey wood, the height of the closed arm walls was 15 cm. Animals were placed in the centre, facing an open arm. The test was recorded with a video camera positioned on a stand at distance of 1 m to the apparatus. Analysis of time

spent on the open arm and closed arm was conducted manually with a timer. Entry into the open arm was recorded only when all four legs of the mouse left the neutral central area. Percentage (%) of the time in the open arm was analysed using two-way ANOVA considering $P<0.05$ as significant.

3.4.5 Fear Memory

Fear memory test is based on the Pavlovian classical conditioning and is a test of learning and memory of emotionally aversive events. In this test, an unconditioned stimulus (usually a foot shock) is accompanied by a conditioned stimulus (usually a tone). The animal learns to associate the aversive memory of US when exposed to the CS alone and thereby, predicts the aversive event responding with fear (resulting in freezing response). A neural circuit comprising, amygdala, hippocampus and PFC regions that are all implicated in schizophrenia are involved in different aspects of this behaviour (Ledoux and Muller, 1997, LeDoux, 2000, Phelps and LeDoux, 2005). Schizophrenia patients have impaired emotional memory, preventing them from decision making and executive functioning and also leading to negative symptoms such as anhedonia(Gard et al., 2007, D'Argembeau et al., 2008, Morris et al., 2009).

Method: Two operant chambers (Kinder Scientific Instruments, Poway, CA) were equipped with a metal grid floor through which a shock of 0.5mA (unconditioned stimulus; US) was delivered and a son-alert to deliver an 85 dB tone (conditioned stimulus; CS). An aversive stimulus (shock of 0.5 mA) as unconditioned stimulus (US) is paired with a conditioned stimulus (CS) (85dB tone) and was given 4 minutes after the

start of the test for two times. CS was given for the duration of 30 seconds and US was given in the last 2 seconds. The conditioning takes a total of 9 minutes. The freezing time for each 30 second bin was calculated based on the automatic rest-time recording using "Motor Monitor" version 5.04 software supplied by the manufacturer.

For assessment of auditory (cued) fear conditioning, mice were tested in a novel chamber of similar size without the shock grid exposed to CS for 3 minutes and freezing was calculated by the same software. The percentage of time freezing was analysed by two-way ANOVA and $P<0.05$ considered significant.

3.5 Neurogenesis

It is now well established that neurogenesis occurs postnatally in certain brain regions, namely the sub- ventricular zone (SVZ) of lateral ventricles and the subgranular zone (SGZ) of the hippocampus (Ming and Song, 2011). Postnatal neurogenesis in hippocampus is involved in cognitive processes such as the learning and memory (Eisch et al., 2008). Further, cytoarchitectural and neurogenesis impairments have been associated with schizophrenia as evidenced by post-mortem studies (Arnold et al., 1991, Akbarian et al., 1993b, Reif et al., 2006). Moreover, studies have shown that genes associated with schizophrenia e.g. DISC-1, neurreguilin-1 and dysbindin-1 are involved in neurogenesis (Le Strat et al., 2009, Mao et al., 2009, Inta et al., 2010, Lee et al., 2011, Nihonmatsu-Kikuchi et al., 2011). On the other hand, postnatal neurogenesis is affected by environmental factors such as infections, cognitive activities, physical activities and stress (van Praag et al., 1999, Eisch et al., 2008, Okun et al., 2010). Specifically, Poly I:C

injected at gestational period has shown impairments in neurogenesis and it has been suggested that TLR-3 stimulation is associated with deficits in neurogenesis (De Miranda et al., 2010).

A new cohort of neonate mice (sandy and C57BL/6 controls (n=4)), were injected with either Poly I:C or saline at PD5,6 and 7. At P7, 2 and 4 hours after the third injection of Poly I:C, all mice were injected with an analogue of thymidine, Bromodeoxyuridine (BrdU; Sigma; 50 mg/kg, i.p.) dissolved in sterile 0.9% saline with 0.7 M NaOH). The solution was vortexed until dissolved. To evaluate fate of the new born cells, four weeks later (considering the time required for maturation of newly born neurons and NeuN expression) mice were assessed.

3.5.1 Tissue processing

Animals were deeply anesthetized with a cocktail of ketamine, xylazine and acepromazine (0.1 ml/100 g), and perfused through the heart with ice-cold phosphate-buffered saline (PBS) followed by 4% formaldehyde in 0.1 M phosphate buffer. Brains were then rapidly removed. Brains were cut using a vibratom(Leica VT1200) into serial 40 μm-thick coronal sections, which were placed in a cryoprotectant solution (glycerol:ethylene glycol:PBS, 3:3:4) and stored at −20°C.

3.5.2 Immunohistochemistry (IHC)

Unless otherwise specified, all IHC incubations were at room temperature. The section-sampling fraction was 1/7.3 sections per brain region per mouse were chosen for assessment. Omitting primary antibodies resulted in an absence of specific staining for the IHC protocol. Rinses with PBS preceded all steps except the addition of primary antibodies. Slices were assessed for immunofluorescent labelling of BrdU and assessment of co-labelling with NeuN in the granular cell layer of the dentate gyrus (dorsal hippocampus) and the granular layer and glomerular layer of the olfactory bulb. IHC were performed using Rat anti-BrdU Ab (Ab Serotec, diluted 1:1000 in 2% NGS in PBS+0.2 % Triton X-100) and affinity purified mouse anti-NeuN Ab (Chemicon, diluted 1:200 in 2% NGS in PBS+0.2 % Triton X-100) and incubated overnight at 4^{o}C. The sections were incubated in the secondary antibodies, goat anti-rat secondary 594(BrdU; red) (Jackson, diluted 1:1500 in 2% NGS in PBS+0.2 % Triton X-100) and goat anti-mouse secondary anti-body 488 (NeuN; green) (Jackson, diluted 1:500 in 2% NGS in PBS+0.2 % Triton X-100) for 90 minutes at room temperature. Sections were mounted on glass slides and cover slipped with Vectashield (Invitrogen).

The immunofluorescent-labellings were assessed using confocal microscopy (Zeiss LSM 510 META). Images were quantified manually using Zeiss LSM image browser software. For the granular cell layer of the dentate gyrus, a grid of 10.5cmx10.5cm was positioned at the most lateral region of the dorsal dentate gyrus. Consistancy in the region evaluated was taken into account throughout the experiment. All the BrdU-labelled and BrdU-NeuN-colabelled cells in the upper crest of the dentate gyrus and the upper half of the

hilus that were placed within that grid were quantified. For the glomerular cell layer of the olfactory bulb, a glomerulus at the position of 5 o'clock was chosen and a grid was positioned around it. The entire immune-labelled cells within that grid were counted. For the granule cell layer of the hippocampus, a 10.5cmx10.5cm grid was positioned in the granule cell region immediately beneath the chosen glomerulus and all of the immune-labelled cells within that grid were quantified.

The mouse behaviours and IHC data were analyzed and treatment and genetic variation were considered as independent variables to study the significance of gene-environment interplay.

3.5.3 Confocal microscopy

Multiple-labeling analyses were conducted at 60X (60x Oil DIC objective) on a Zeiss LSM510 Meta confocal microscope equipped with an Axiovert 200 M stand and motorized stage (Carl Zeiss Canada), using 543 nm, and 633 nm wavelength lasers. Images were obtained using the Zeiss Aim software package (Carl Zeiss Canada), at a pixel size of 0.11 μm for x and y, a scan average of ≥4 frames, a pixel dwell time of ≥3.20 μs, and sampled at an interval of 2 μm. Images were not modified with exception of overall brightness and contrast. For quantitative analyses, images were manually counted using Zeiss LSM image browser software as described above.

4. RESULTS

4.1 Behaviour

4.1.1 Spontaneous locomotion and stereotypy

The results from spontaneous locomotor activity (Fig. 1a and 1b) has demonstrated a significant main effect of Sdy (-/-) genotype on locomotion (F (2,4 7)= 4.115, $p < 0.05$*). Sdy (-/-) mice showed higher locomotor activity in comparison to Sdy(+/-) and the WT animals. However, no interaction effect was observed between genotype and neonatal Poly IC treatment (F(2,47)= 0.02001, $p > 0.05$). Further, separating the male and females, the main effect of the Sdy (-/-) was observed only in the male group (F (1, 21)= 14.19, $p < 0.05$) and not in the female group of mice (F (1,12)= 0.009, $p > 0.05$) (Fig. 1c and 1d). In terms of stereotypy, no significant main effect of genotype (F (2,53)=0.2711, $p > 0.05$), treatment (F (1,53)= 1.328, $p > 0.05$)or interaction(F (2,53)= 0.575, $p > 0.05$) was observed(Fig.1e).

4.1.2 Novel object recognition memory

ANOVA of the results demonstrate that no significant main effect of genotype(F (2,44)=1.095, $p > 0.05$), treatment (F (1,44)=0.219, $p > 0.05$), or their interaction (F (2,44)=0.694, $p > 0.05$), exists on percentage of time spent on exploring the novel object. This suggests that neither Sdy (-/-) mutation nor neonatal Poly I:C treatment significantly affected adult memory(Fig. 2a). Analysing male and female mice separately, no significant main effect of genotype (male: F (1,14)=0.436, $p > 0.05$) (female: F (1,14)=

0.046, $p > 0.05$), treatment (male: F (1,14)=0.138, $p > 0.05$) (female: F (1,14)= 0.250, $p > 0.05$) or their interaction (male: F (1,14)=0.005, $p > 0.05$) (female: F (1,14)= 2.243, $p > 0.05$) was observed in terms of novel object exploration. (Fig. 2b and 2c)

4.1.3 Prepulse inhibition of acoustic startle (PPI)

There was no overall significant difference in prepulse inhibition or the startle response between the groups. The results demonstrate that no significant main effect of genotype(F (1,14)=0.753, $p > 0.05$), treatment (male: F (1,14)=1.000, $p > 0.05$) or their interaction(F(2,53)= 1.770, p > 0.05), exists on % PPI (Fig. 3a and 3b). Analysing male and female mice separately, no significant main effect of genotype(male: F (1,13)=0.005, $p > 0.05$) (female: F (1,13)= 0.208, $p > 0.05$), treatment(male: F (1,13)=1.388, $p > 0.05$) (female: F (1,13)= 2.012, $p > 0.05$) or their interaction(male: F (1,13)=0.193, $p > 0.05$) (female: F (1,13)= 0.450, $p > 0.05$) was observed neither in the males nor females in terms of %PPI.(Fig.3c and 3d)

4.1.4 Elevated plus maze

Overall, the animals in all groups spent more time in the closed arm than the open arm and such avoidance was expected in the context of an anxiety inducing condition. The x axis in the graphs (Fig.4a) represents the percentage of the time in the open arm.and the results demonstrate that no significant main effect of genotype (F (2,42)= 1.168, $p > 0.05$) , treatment (F (1,42)= 0.045, $p > 0.05$) or their interaction (F (2,42)= 1.156, $p > 0.05$

) exists on the percentage of time spent on the open arm. Analysing male and female mice separately, no significant main effect of genotype (male: F (1,12)=1.262, $p > 0.05$) (female: F (1,14)= 0.618, $p > 0.05$) , treatment (male: F (1,12)=0.631, $p > 0.05$) (female: F (1,14)= 1.373, $p > 0.05$) or their interaction (male: F (1,12)=0.449, $p > 0.05$) (female: F (1,14)= 1.117, $p > 0.05$) was observed neither in the males nor females in terms of percentage of time spent on the open arm(Fig.4b and 4c).

4.1.5 Fear Memory

Animals in all groups showed higher freezing in the second trial of shock during the training phase (day1) indicating that all the mice learned CS-US association equally well (Fig. 5a). The data on conditioned cued (tone) memory demonstrates that no significant main effect of genotype(F (2,30)= 1.345, $p > 0.05$), treatment(F (1,30)= 0.162, $p > 0.05$) or their interaction(F(2,30)= 1.106, p > 0.05) exists on the percentage of freezing. (Fig.5b)

4.2 Neurogenesis

4.2.1 Hippocampus

To assess the effect of the interaction on the fate of the new born cells in the dentate gyrus during the time of immune challenge, we looked at the immunostaining of BrdU-labelled cells in this region. Many BrdU-labelled cells were observed in the SGZ, the the granular cell layer(mostly in vicinity of the SGZ) and sparcely in the hilus. (Fig.6). The

relative abundance of the newborn neurons compared to what is seen in adult dentate gyrus neurogenesis is expected considering that the immunolabelled cells were born in the first postnatal week.

The upper crest of the most lateral region of the dentate gyrus and the upper half of the hilus was placed within a grid (10.5cmx10.5cm) and the BrdU-positive and BrdU-NeuN-colabelled cells were quantified.The results demonstrate that no significant main effect of genotype(F (1,11)=0.412, $p > 0.05$), treatment (F (1,11)= 0.902, $p > 0.05$)or their interaction(F (1,11)= 0.383, $p > 0.05$) exists on the number of BrdU-positive cells in the granular cell layer (region described above) of the dentate gyrus four weeks after the treatment with Poly I:C(Fig.7a).Further, no significant main effect of genotype(F (1,11)= 0.001, $p > 0.05$), treatment (F (1,11)= 0.163, $p > 0.05$)or their interaction(F (1,11)= 0.064, $p > 0.05$) exists on the percentage of BrdU-positive cells in the granular cell layer of the dentate gyrus that expressed NeuN four weeks after the treatment with Poly I:C (Fig. 7b).

4.2.2 Olfactory bulb

We also looked at the effect of the interaction on the fate of the new born cells that reach the olfactory bulb. Immunostaining of BrdU-labelled cells and BrdU-NeuN colabelling was assessed. BrdU-labelled cells were observed in the glomerular cell layer (quantifying the glomerulus at position of 5 o'clock) and granule cell layer (positioned within a grid of 10.5cmx10.5cm right beneath the selected glomerulus) of the olfactory bulb (Fig. 8a

and 8b). There was also relatively abundant number of BrdU-positive cells dispersed in other layers of the olfactory bulb which is expected considering the age of the animals.

The results demonstrate that a significant main effect of Sdy/Sdy genotype exists on the number of BrdU-positive cells comparing the representative glomerulus in the glomerular layer of the olfactory bulb four weeks after the treatment with Poly I:C (F (1,11)= 5.388, $p < 0.05$*)(Fig. 9a). However, no significant main effect of early immune-activation (F (1,11)= 0.183, $p > 0.05$) or interaction (F (1,11)= 0.713, $p > 0.05$) exists on the number of BrdU-NeuN positive cells in the same glomerulus.(Fig.9b)

The granular cell layer located right beneath the selected glomerulus in each slide was assessed. The BrdU- labelled and BrdU-NeuN colabelled cells were quantified. The results on granular cell layer similarly demonstrate that no significant main effect of genotype(F (1,11)= 0.909, $p > 0.05$), treatment (F (1,11)= 1.187, $p > 0.05$) or their interaction (F (1,11)= 1.358, $p > 0.05$) exists on the number of BrdU-positive cells in the granular cell layer of the olfactory bulb four weeks after the treatment with Poly I:C. (Fig.10a)

However, the percentage of BrdU-NeuN positive cells were unaffected by genotype(F (1,11)= 0.077, $p > 0.05$), treatment(F (2,30)= 0.302, $p > 0.05$) or their interaction (F (1,11)= 0.001, $p > 0.05$) (Fig.10b).

5. DISCUSSION

This study aimed to examine an important issue in the pathogenesis of schizophrenia, namely the interaction between genetic susceptibility and environmental insults. Evidence on the pathogenesis of schizophrenia indicates a role for both genetic and environmental factors. We examined the possible interactive effects of early (neonatal) immune challenge by Poly I:C and mutation in dysbindin-1 gene on schizophrenia-related behaviours. Also, we looked at the immediate effects of this interaction on the fate of the cells migrating to three regions of the brain the granular cell layer of the dentate gyrus in the hippocampus and the granule layer and the glomerular layer of the olfactory bulb.

As referred to in the introduction, Edwards et al have repoted an interaction between dysbindin-1 gene and IL3 gene that increases the risk of schizophrenia (Edwards et al., 2008). IL3 is a cytokine secreted by the immune cells. Therefore, we found it plausible to look at the interaction between this gene and early immune challenge.

Our study reveals that immune-activation using our chosen dosage/time of Poly I:C in mice carrying dysbindin-1 mutation does not result in pronounced or new schizophrenia-related phenotypes in the selected number of behavioural and neurobiological end points.

Gene-environment interaction exists not only in the pathogenesis of schizophrenia but also in other psychiatric disorders such as depression and bipolar disorder (Thapar et al., 2007, Heim and Binder, 2012). Therefore, models of measured gene-environment interaction serves the purpose of studying the overlapping behavioural and pathophysiological aspects in mental disorders.

The lack of a positive main effect of the mutation on behaviours other than spontaneous locomotion was unexpected. To our surprise, the main effect of dysbindin-1 mutation was not observed in the other behaviours assessed. This could be explained by the fact that the background of the dysbindin-1 mutant mice in this study was C57Bl/6 and most studies done on dysbindin-1 mutant mice were on DBA2/J background which demonstrate deficits in fear memory, spontaneous locomotion and novel object recognition memory. It is possible that mice on DBA/2J background contain other mutations that result in the occurrence of the named behavioural deficits. In the novel object recognition test, parameters such as the characteristics of the objects, intensity of the light in the room may explain the difference in the results.

In another study on dysbindin-1 mutants bred on C57BL/6 background, PPI deficit has been reported. This study was conducted in different prepulse intensities (i.e. 74, 78, 82, 86 and 90 dB) than ours. The significant main effect of the homozygous mutation in dysbindin-1 (-/-) was observed in the prepulse intensities of 74 , 86 and 90(Papaleo et al., 2012). However, in our experiment, prepulse intensities of 76 dB, 79 dB, 82 dB, 85 dB were applied and no significant difference was observed between the groups.

On the other hand, Poly I:C- induced immune-activation in neonates did not result in behavioural deficits in adult mice, as far as the behaviours we assessed were concerned. This was in contrast to the findings by ibi et al. (Ibi et al., 2009) who had observed anxiety-like behavior , sensorimotor gating deficits and impairments in object recognition memory and social behavior in the neonate mice treated with Poly I:C compared to the saline-treated control group. We think that lack of a behavioural deficit in our Poly I:C treated mice stems from the lower dosage of Poly I:C(4mg/kg versus 5

mg/Kg), shorter period of treatment (3 days versus 5 days) and later start of treatment (P5 versus P3).

Therefore, quantification of the immune-response to the administered dosage of Poly I:C could determine the adequacy of the dosage in stimulating immune-activation. Apart from that, since deficits in spatial learning and memory have been observed in dysbindin-1 mutant mice (Cox et al., 2009), we are curious to see if the interaction would result in more robust deficits in the spatial learning and memory. Our findings in regards to lack of an interactive effect between our chosen genetic mutation and environmental insult is not surprising in the context of a complex psychiatric disorder such as schizophrenia where multiple genetic factors interact with multiple environmental factors to induce certain phenoypes. Therefore, the results help us in knowing that the chosen factors do not interact in terms of our selected endpoints. However, this does not falsify the gene-environment hypothesis in pathogenesis of schizophrenia and other complex psychiatric illnesses.

This study shows that Sdy/Sdy mice have lower number of new born cells in the glomerular layer of the olfactory bulb. The glomerular layer is the outer layer of the olfactory bulb. Each Glomerulus is a spherical to oval-shaped structure surrounded by Juxtaglomerular cells, comprised of neurons and glial cells. This layer is located at the region where olfactory nerve makes synapses with the mitral, periglomerular and tufted cells (Valverde et al., 1992, Kosaka et al., 1998, Wachowiak and Shipley, 2006). Valverde et al investigated the development of the olfactory nerve and reported that the olfactory glomeruli is the only structure that continues undergoing development during the first postnatal weeks to the end of the first postnatal month(Valverde et al., 1992).

This report notes the susceptibility of the glomerular layer during the first postnatal week. The new born neurons that migrate to the glumerular layer differentiate into GABAergic interneurons and dopaminergic neurons(Betarbet et al., 1996). Each group of glomeruli are basically specific to a type of odor and odor receptors in the olfactory epithelium. This provides a mapping for odors in the glomerual layer(Uchida et al., 2000, Xu et al., 2000, Meister and Bonhoeffer, 2001). Deficits in olfactory-associated functions such as olfactory identification and discrimination have been previously reported in schizophrenia subjects (Hurwitz et al., 1988, Malaspina et al., 1994, Brewer et al., 1996, Moberg et al., 1997, Arnold et al., 2001, Rioux et al., 2005). On the other hand, Sandy mice have not demonstrated deficits in olfaction or the olfactory habituation/dishabituation tasks (Talbot, 2009, Papaleo et al., 2012). Also, the direct and indirect connections between the olfactory bulb and the hippocampus could suggest common roles for example in the spatial learning and memory deficits observed in sandy mice (van Groen and Wyss, 1990, de la Rosa-Prieto et al., 2009). It is also possible that the lower number of new-born cells reaching the glomerular layer is due to a decrease in radial migration of the new-born cells from RMS to the glomerular layer, once they reach the olfactory bulb.

6. CONCLUSION

In conclusion, this project was primarily designed to show that Poly I:C injection in neonate Sandy mice would induce either greater (interactive) effect on cognition and neurogenesis or result in a new phenotype not present in each model individually.

A number of selected schizophrenia-relevant behavioral assessments were tested to capture the possible interaction between dysbindin-1 mutation and Poly I:C.

Neurogenesis in the SGZ of the hippocampus is of interest in schizophrenia due to the important role of hippocampus in cognition and also the presence of pathologies in hippocampus in schizophrenia subjects. SVZ neurogenesis, although is not well clear to be linked to schizophrenia, was be studied since it could shed light to the effect of dysbindin1-Poly I:C interaction on common pathways involved in neurogenesis. In my study due to limitations in the number of genetically mutant mice, all the groups assessed contained both male and female mice which further resulted in large error bars in a number of behavioural tests. When separating males and females, we still did not observe the significant main effect of the genotype, treatment or interaction. Furthermore, the limitation on the number of mutant mice prevented us from examining the effects of other immune activators such as LPS and CpGDNA and also other behavioural (e.g. spatial learning and memory and social interaction) and pathophysiological (e,g. Density of different subgroups of DA, NMDA receptors and parvalbumine neurons, dendritic arborisation and spine density) endpoints. We further find it interesting to look at the possible triggering effect of the immune activators in young adult animals, as the neonatal immune activation might render the individual susceptible to later immune-

challenge. Exploration with higher doses of the immune activator and/or different timeline of treatment is needed to fully test GxE hypothesis linking immune activation and dysbindin-1 gene . Therefore, the hypothesis that genetic and environmental factors could interact to produce a phenotype not affected by dysbindin-1 genotype or neonatal infection is still possible . In the path to explore the potential interactive effect of dysbindin-1 and early immune-activation, this study helps to narrow the future experiments to different possibilities of experiment variables and measure.

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8. FIGURES AND TABLES

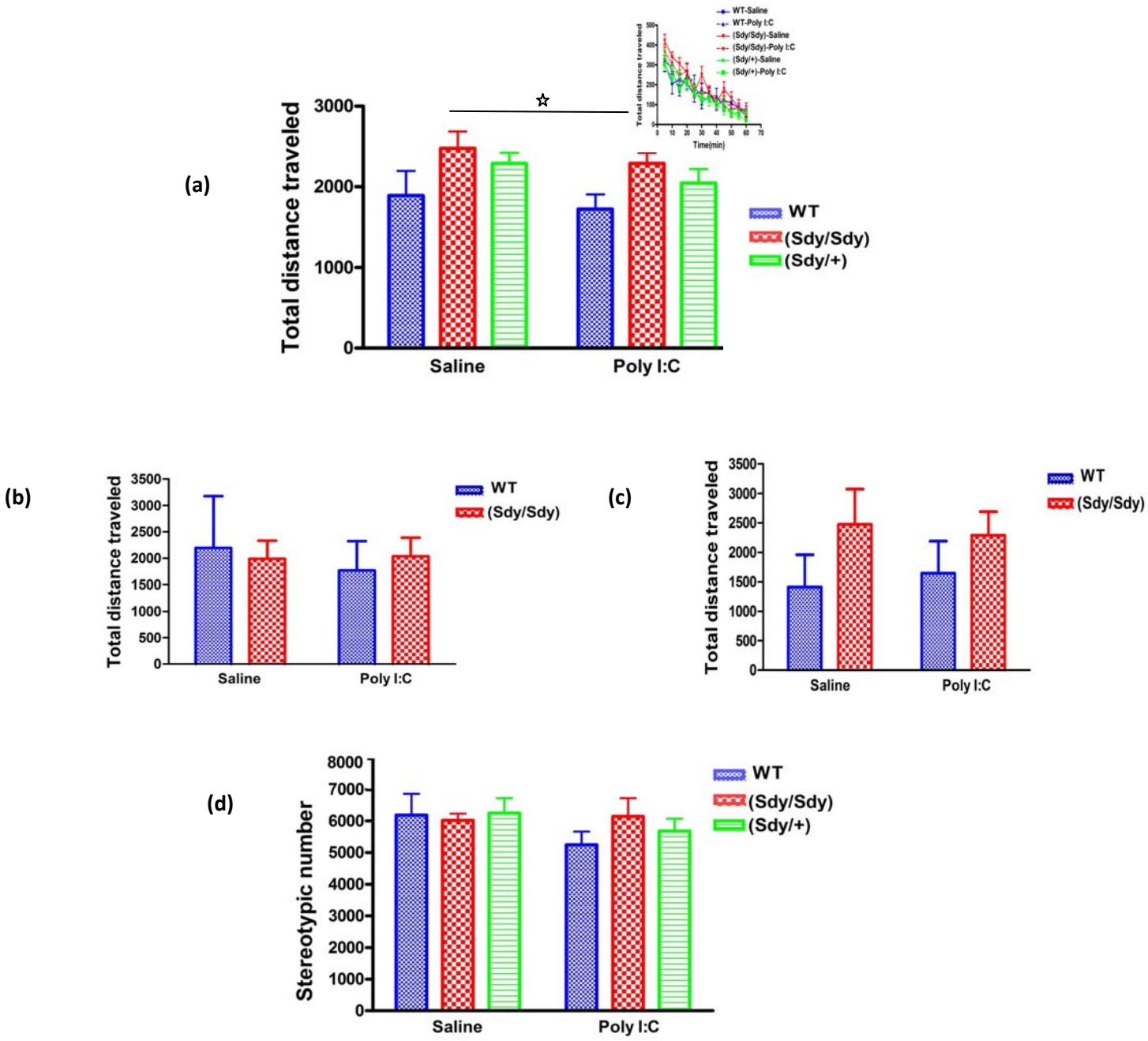
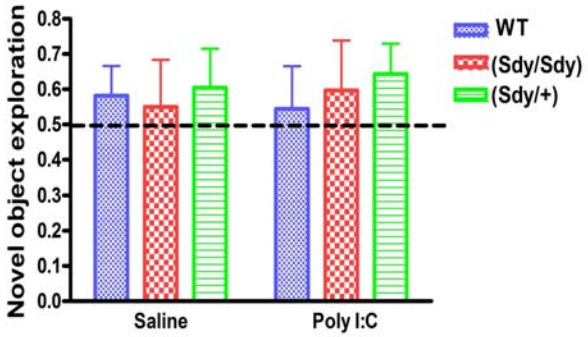


Fig. 1. Spontaneous locomotor activity. (a ,inset)) A significant main effect of Sdy/Sdy genotype on locomotion is observed ($p < 0.05$*). No main effect of the interaction was observed between genotype and treatment. Fig.1a demonstrates the total time travelled and Fig.1a(inset) shows Locomotor activity in novel environment measured at 10 min intervals. (c,d) Separating male and females, the main effect of the Sdy/Sdy genotype was observed only in the male group ($p < 0.05$#) and not in the female mice. (e) Stereotypy remained unaffected by genotype, treatment or interaction.

(a)



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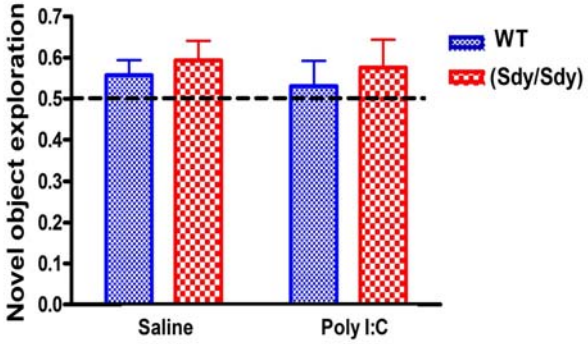

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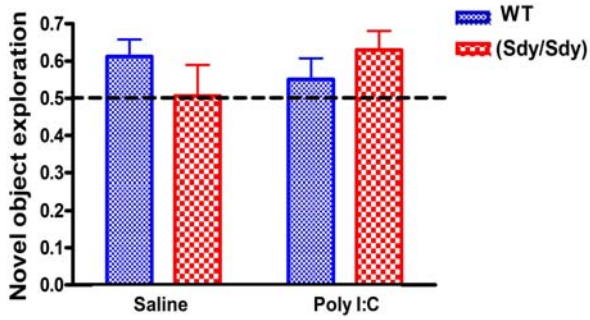


Fig. 2. Novel object recognition memory. (Fig. 2a) ANOVA of the results demonstrate no significant main effect of genotype, treatment, or their interaction on percentage of time spent on exploring the novel object. Neither Sdy (-/-) mutation nor neonatal Poly I:C treatment significantly affected adult memory. (Fig.2b and 2c) Analysing male and female mice separately, no significant main effect of genotype, treatment or their interaction was observed in terms of novel object exploration.

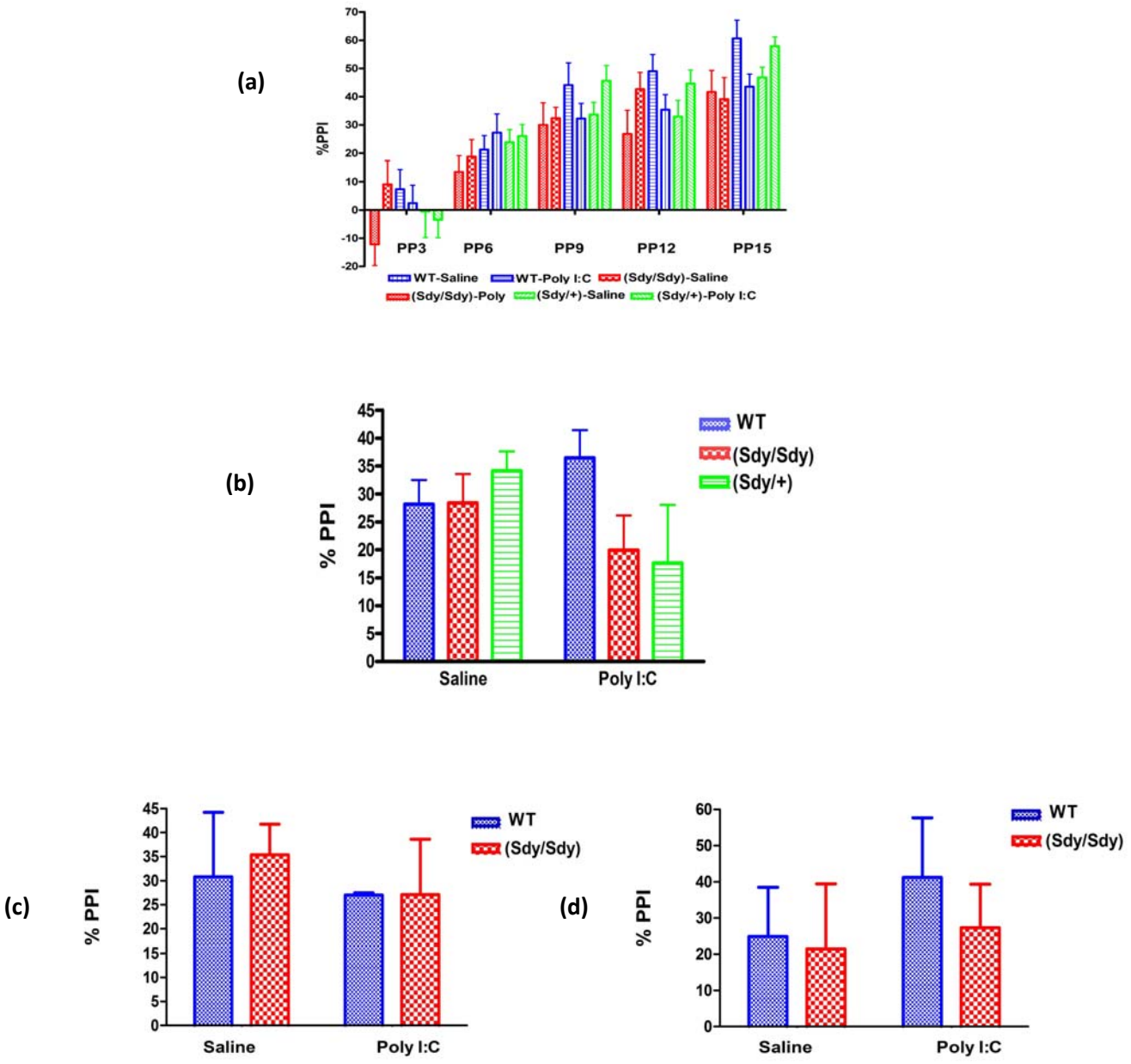


Fig. 3. Prepulse inhibition of acoustic startle (PPI). (Fig. 3a and 3b)There was no overall significant difference in prepulse inhibition or the startle response between the groups. The results demonstrate no significant main effect of genotype, treatment or their interaction exists on % PPI. (Fig. 3c and 3d) Analysing male and female mice separately, no significant main effect of genotype, treatment or their interaction was observed neither in the males nor females in terms of %PPI.

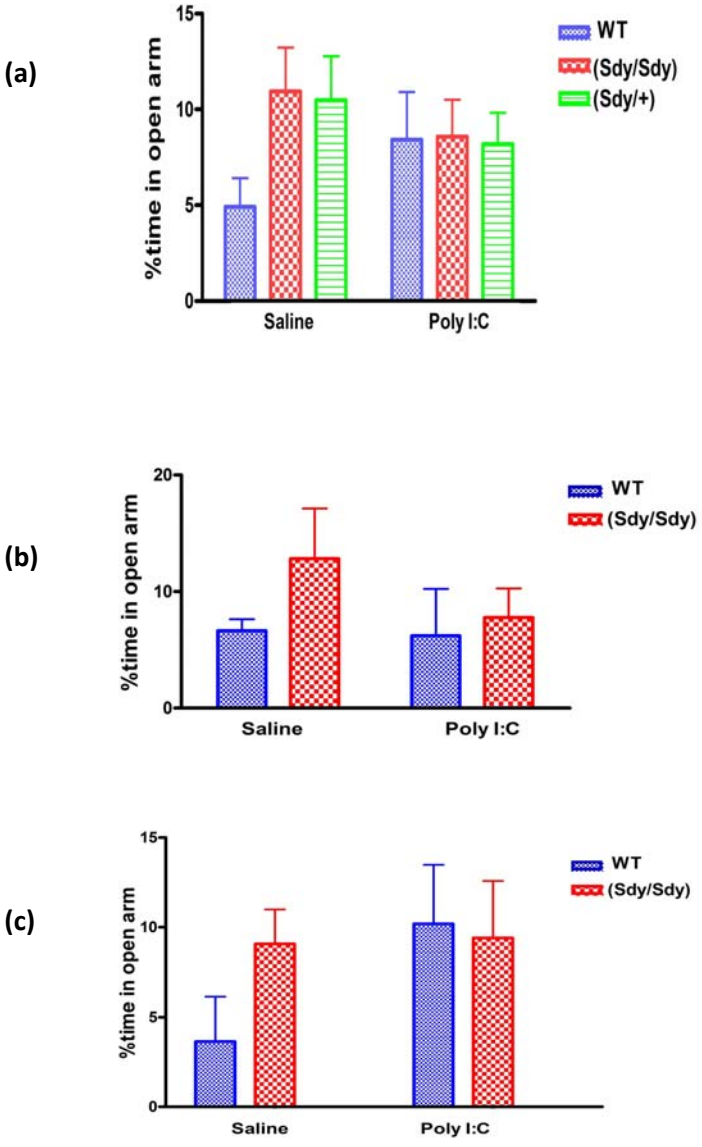
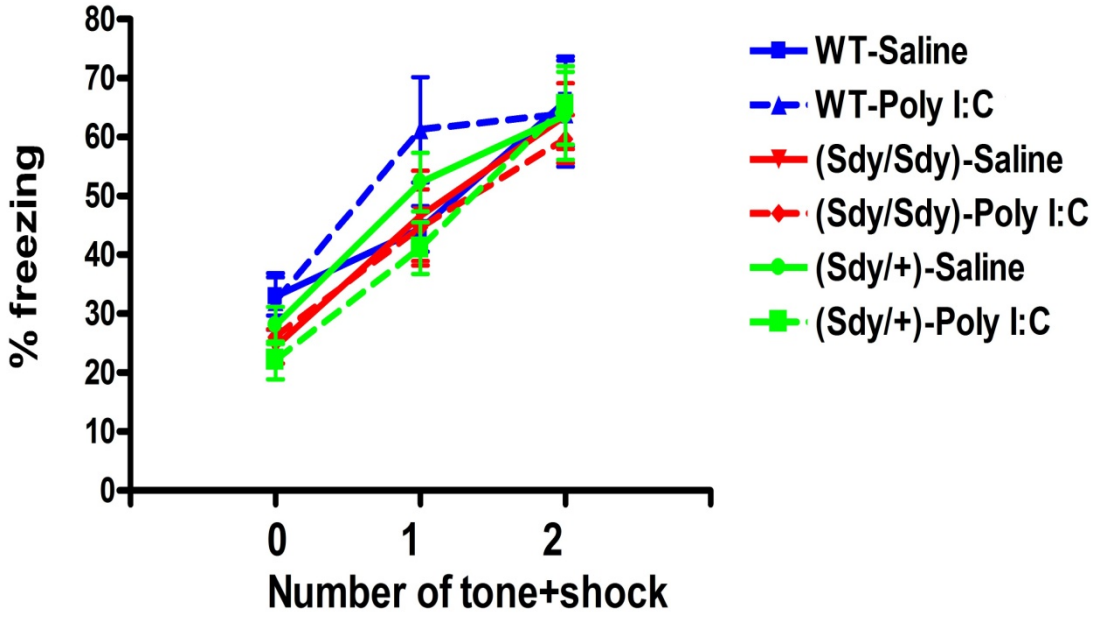


Fig. 4. Elevated plus maze. Animals in all groups spent more time in the closed arm than the open arm. The x axis in the graphs represents the percentage of time in the open arm. (Fig. 4a)The results demonstrate that no significant main effect of genotype, treatment or their interaction exists on the percentage of time spent on the open arm. (Fig. 4b and4c) Analysing male and female mice separately, no significant main effect of genotype treatment or their interaction was observed neither in the males nor females in terms of percentage of time spent on the open arm.

(a)



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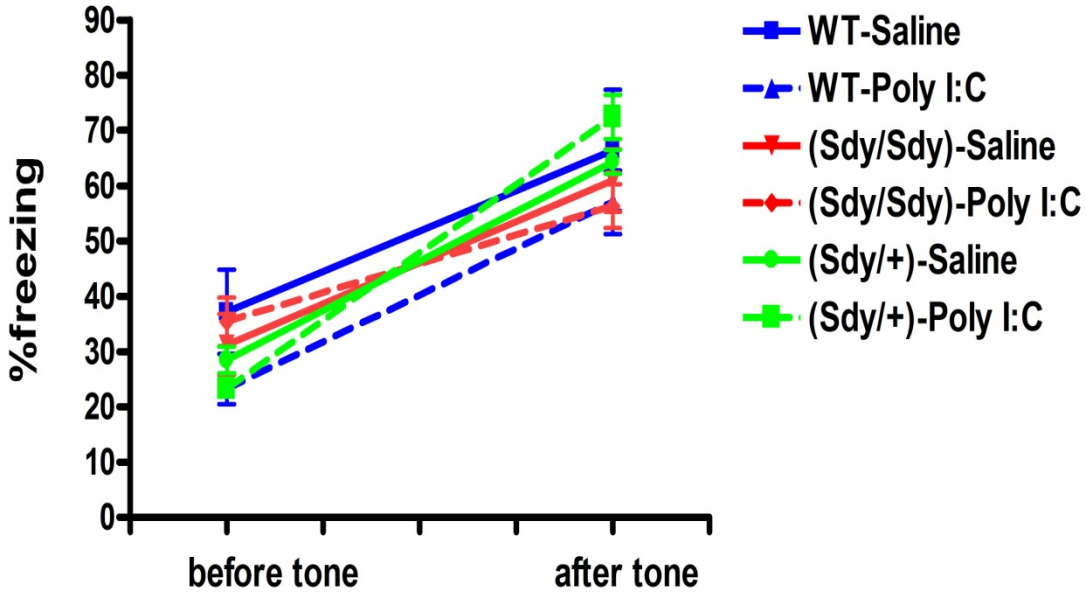


Fig. 5. Fear memory. (Fig.5a)Animals in all groups showed higher freezing in the second trial of shock during the training phase (day1) indicating that all the mice learned CS-US association equally well. (Fig. 5b)The data on conditioned cued (tone) memory shows no significant main effect of genotype, treatment or their interaction exists on the percentage of freezing.

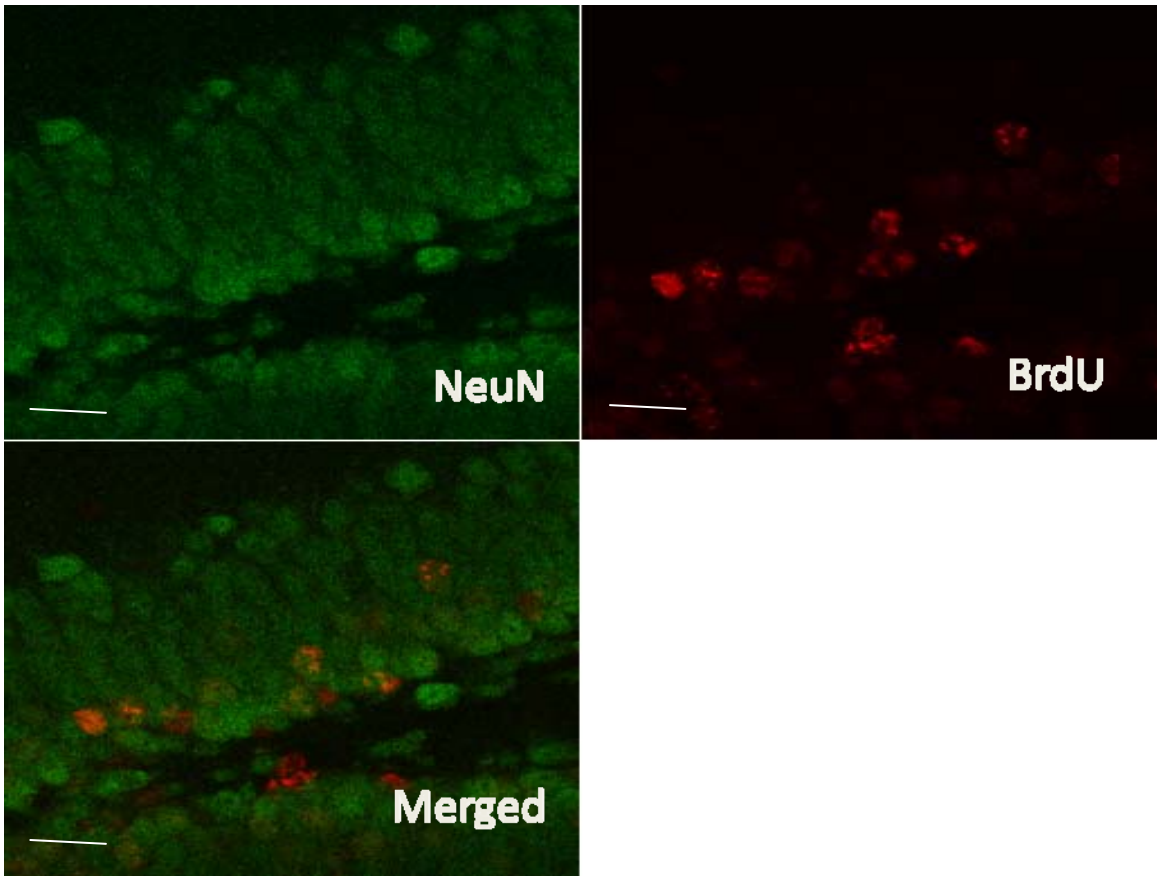
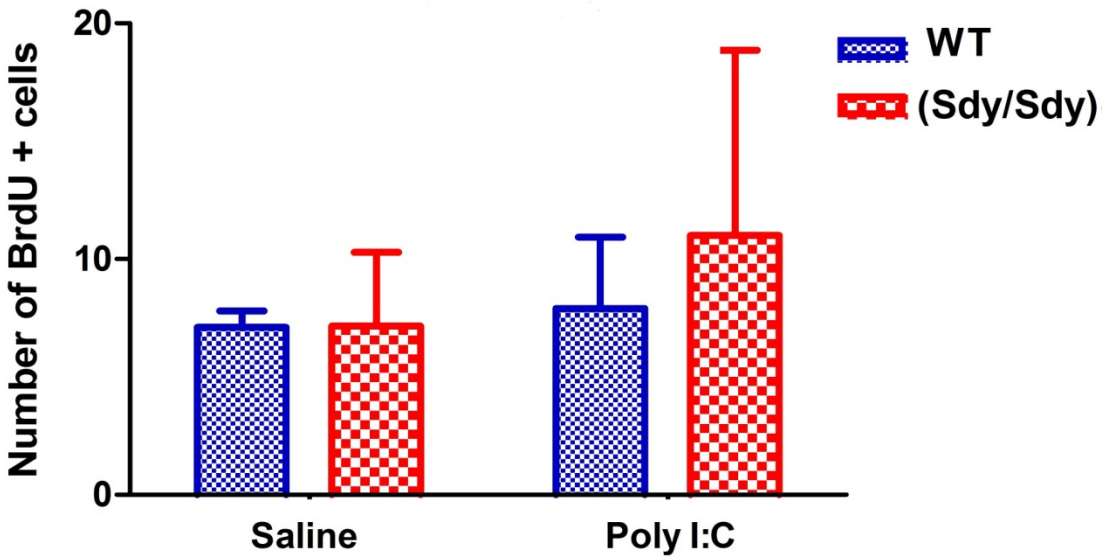


Fig. 6. BrdU and BrdU-NeuN colocalization. Confocal micrographs (60X, oil immersion) of the dentate gyrus. BrdU-positive cells (red) and NeuN (green). In the dorsal hippocampus, the upper crest of the most lateral region of the dentate gyrus and the upper half of the hilus was placed within a grid (10.5cmx10.5cm) and the BrdU-positive and BrdU-NeuN-colabelled cells were quantified.(Scale bar, 20µm)

(a)



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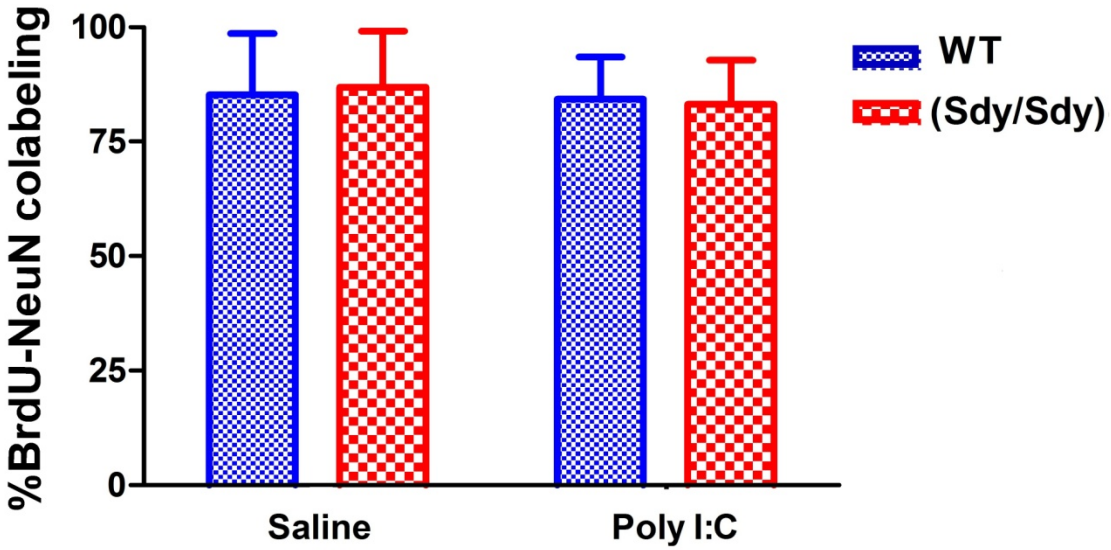
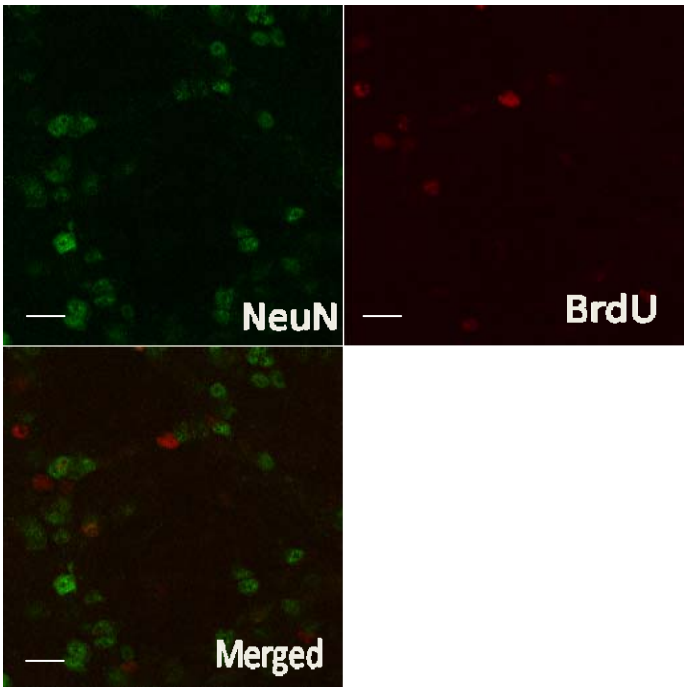


Fig. 7. Cell fate in the Dentate gyrus.(Fig. 7a)The results demonstrate that no significant main effect of genotype, treatment or their interaction exists on the number of BrdU-positive cells in the granular cell layer (region described in Fig 6) of the dentate gyrus four weeks after the treatment with Poly I:C. (Fig. 7b) No significant main effect of genotype, treatment or their interaction exists on the percentage of BrdU positive cells in the granular cell layer of the dentate gyrus that expressed NeuN four weeks after the treatment with Poly I:C.

(a)



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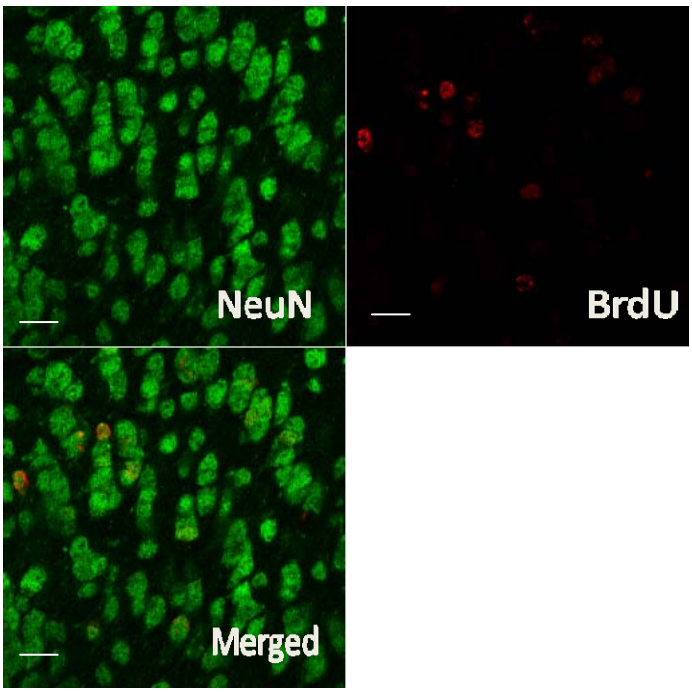

Fig. 8. BrdU and BrdU-NeuN colocalization in the Olfactory bulb. Confocal micrographs (60 X, oil immersion) of a glomerulus (Fig. 8a) and the granule cell layer (Fig. 8b) in the olfactory bulb. BrdU-positive cells (red) and NeuN (green). BrdU-labelled cells were observed in the glomerular cell layer (quantifying the glomerulus at position of 5 o'clock) and granule cell layer (positioned within a grid of 10.5cmx10.5cm right beneath the selected glomerulus) of the olfactory bulb. (Scale bar, 20µm)

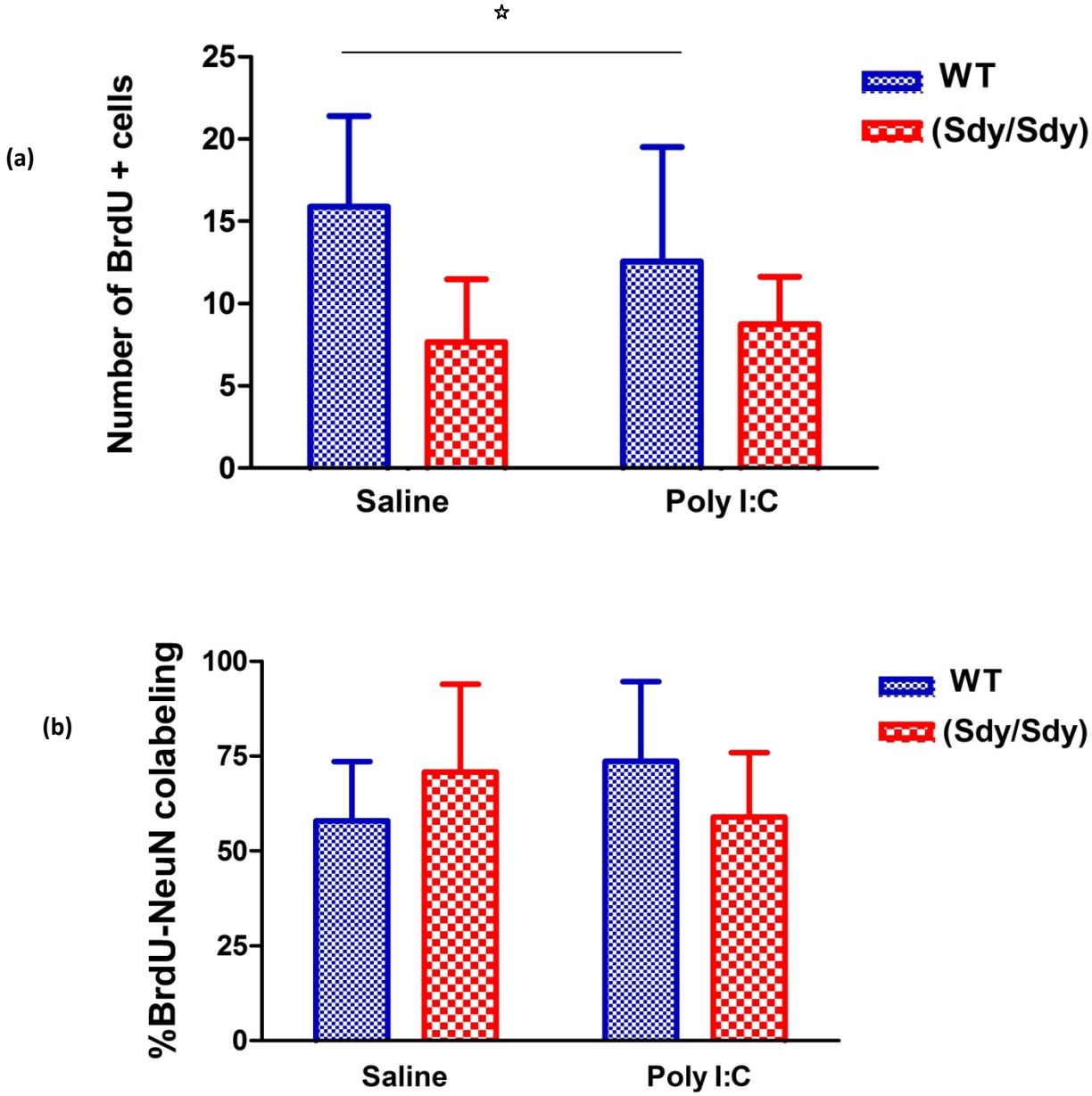

Fig. 9. BrdU and BrdU-NeuN colocalization in the glomerular cell layer of the Olfactory bulb. (Fig. 9a)The results demonstrate that a significant main effect of Sdy/Sdy genotype exists on the number of BrdU positive cells comparing the representative glomerulus in the glomerular layer of the olfactory bulb four weeks after the treatment with Poly I:C/BrdU($p < 0.05$*). (Fig. 9b) No significant main effect of early immune-activation or interaction exists on the number of BrdU-NeuN positive cells in the same glomerulus.

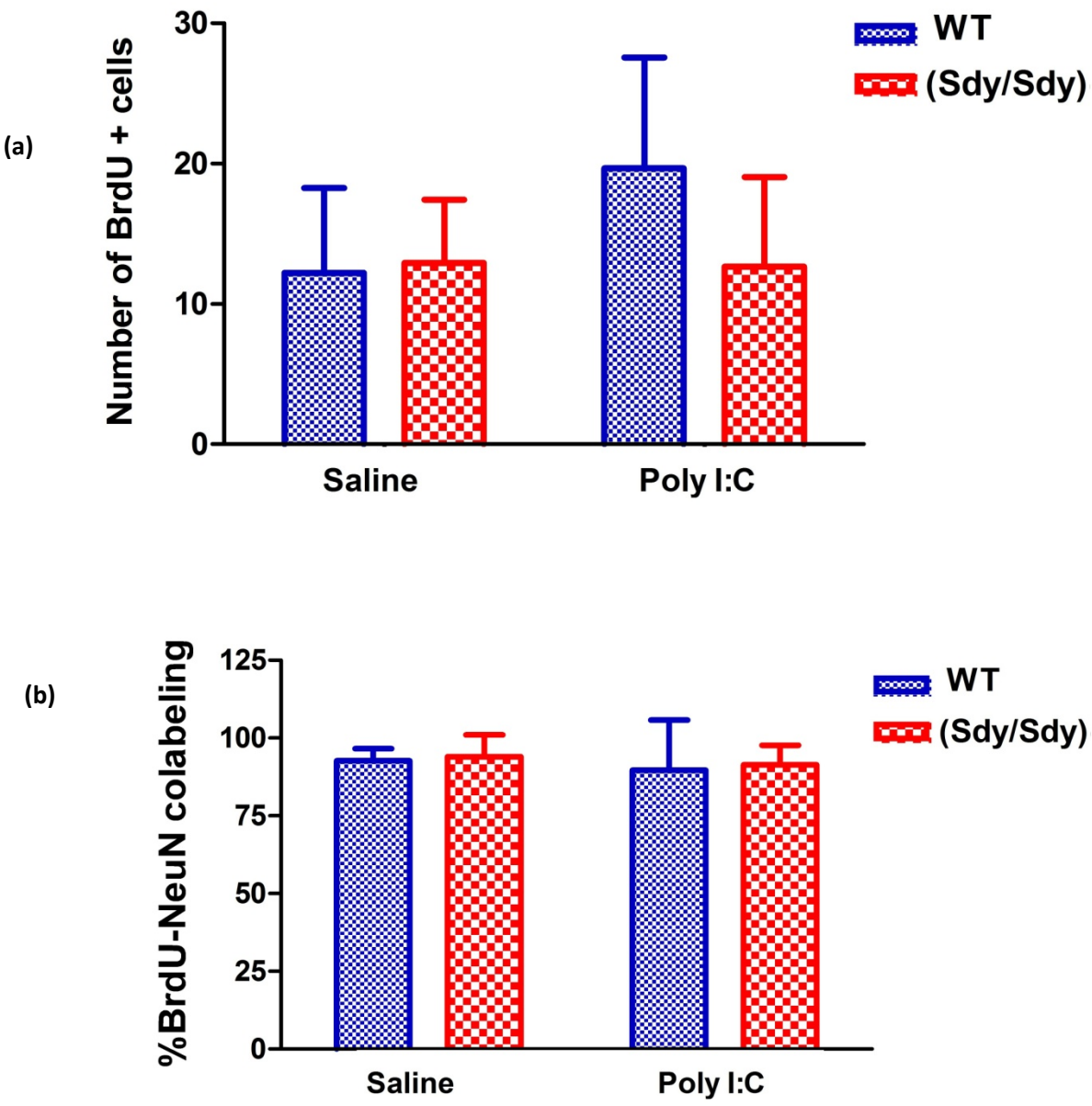

Fig.10. BrdU and BrdU-NeuN colocalization in the glomerular cell layer of the Olfactory bulb. The granular cell layer located right beneath the selected glomerulus in each slide was assessed. (Fig.10a)The results on granular cell layer similarly demonstrate that no significant main effect of genotype, treatment or their interaction exists on the number of BrdU- positive cells in the granular cell layer of the olfactory bulb four weeks after the treatment with Poly I:C. (Fig.10b)The percentage of BrdU-NeuN positive cells were unaffected by genotype, treatment or their interaction.