Role of a PTP1B pathway in the neuropsychiatric expression of a mouse maternal immune activation model

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Abstract

Activation of the immune system in gestating mothers has been identified as an important environmental risk factor for neuropsychiatric disorders. Maternal immune activation (MIA) animal models have been used to explore how the maternal immune system may cause expression of pathophysiology in offspring. Protein tyrosine phosphatase (PTP1B) is recruited during inflammation and its regulatory proteins are modulated in MIA. Disrupted regulation of PTP1B has been linked to mental disorders such as Rett Syndrome and anxiety. We asked if ablating neuronal PTP1B could protect from the expression of some neuropsychiatric phenotypes that appear in MIA models. In our MIA model induced with poly I:C injection at gestational day 9.5, we observed increased locomotion and sensorimotor gating and reduced anxiety in 3-month-old male offspring while females showed decreased sensorimotor gating. These effects were not replicated in PTP1B KO mice indicating a role of PTP1B in affecting locomotion and anxiety level in MIA. This model promotes a more balanced understanding of MIA and introduces PTP1B as a player in MIA-induced behaviour changes.

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AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazoleprionic acid
AP-1	Activator Protein 1
ASD	Autism spectrum disorder
Asp	Aspartic acid
ASR	Acoustic startle response
BBK	Beam break test
BDNF	Brain derived neurotrophic factor
BLA	Basolateral amygdala
CamK2α	Calcium/calmodulin dependant protein kinase II alpha
CD	Cluster of differentiation
COPD	Chronic obstructive pulmonary disease
CRH	Corticotropin-releasing hormone
Cys	Cysteine
D2R	Dopamine Receptor 2
DAMP	Damage-associated molecular pattern
DISC1	Disrupted in schizophrenia-1
DNA	Deoxyribonucleic acid
DSM	Diagnostic and Statistical manual of Mental Disorders
E	Embryonic day
EPM	Elevated plus maze
EPSC	excitatory postsynaptic current
FST	Foot shock test
GABA	gamma-aminobutyric acid
GD	Gestational day

Gln	Glutamine
GPx-1	glutathione peroxidase
GSK3β	Glycogen synthase kinase 3β
GWAS	Genome-wide association study
HLA	Human leukocyte antigen
HSP	Heat shock protein
IL	Interleukin
IFN	Interferon
IRF	Interferon regulatory factor
IRS	Insulin receptor substrate
IKK	I kappa beta kinase
KO	Knock out
LMO4	LIM Domain Only 4
LPS	Lipopolysaccharide
MAPK	mitogen-activated protein kinase
MECP2	methyl-CpG-binding protein 2
MHC	Major histocompatibility complex
MIA	Maternal immune activation
MyD88	myeloid differentiation primary response gene 88
nAChR	Nicotinic acetylcholine receptor
NLRP3	NOD-like receptor family member NOD- LRR- and pyrin domain-containing 3
NKC	Natural killer cell
NF-κB	kappa-light-chain enhancer of activated B cells
NMDA	N-methyl-D-aspartate
NR1	NMDA receptor subunit 1

Nurr	nuclear receptor related
OF	Open field
PAMP	Pathogen-associated molecular patterns
PCP	Phencyclidine
PFC	Prefrontal cortex
Poly I:C	Polyinosinic-polycytidylic acid
PPI	Prepulse inhibition
PRR	Pathogen recognition receptor
PTP1B	Protein tyrosine phosphatase 1
PYK2	Neuronal protein tyrosine kinase
RGC	Radial glial cell
RNA	ribonucleic acid
RNS	Reactive nitrogen species
RORγt	Retinoic acid receptor-related orphan nuclear receptor gamma t
ROS	Reactive oxygen species
SI	Social interaction test
Th	Helper T cell
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRIF	TIR-domain-containing adapter inducing interferon-β
TrKB	Tropomyosin receptor kinase B
TSC	Tuberous sclerosis complex
WT	Wildtype
YBX1	Y-box binding protein 1
YM	Y-maze

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Introduction

Schizophrenia

Schizophrenia is a severe mental disorder that affects 0.7% of the population and does not discriminate against race or sex [1]. The variety of symptoms have been classified intro three categories: positive (hallucinations, delusions), negative (flat affect, social withdrawal, and lack of speech, motivation and pleasure), and cognitive symptoms (executive function deficits affecting working memory and attention control) [2]. Many unique combinations of these symptoms may occur in any given individual. Yet, these individuals share a disorder together that impacts their quality of life and that of their support group not without repercussions on society [1].

Presently, most antipsychotics are categorized as first-generation (typical) or second-generation (atypical) and all target dopamine receptor type 2 (D2). The latter also targets serotonin receptors. While they help alleviate positive symptoms, these antipsychotics have adverse effects and little to no efficiency in treating negative and cognitive deficits [3]. There is therefore a need for improved treatment. To elucidate the complex pathological pathways of schizophrenia and propose new efficient pharmacotherapies, many animal models of neuropsychiatric disorders have been established.

Some of these are genetic models based on the genome-wide association study (GWAS) [4], especially via the deletion of genes where there is a high correlation between mutation and disorder. For example, Chromosome 22q11.2 microdeletion causes velocardiofacial syndrome and is linked with schizophrenia-like syndrome in a third of cases but with other neuropsychiatric disorders in

other cases, sometimes within the same family [5]. Homologous mice models are used but not without debate of their construct validity. Another common model based on a penetrant human mutation includes disruption of Disrupted in schizophrenia-1 (Disc-1) gene. DISC-1 inhibits Glycogen synthase kinase 3 β (GSK3β) to regulate neuronal proliferation and assist neuron migration, axon elongation, and synaptic function [6]. Different variations of this model have been established, yet again, without specificity for schizophrenia [7].

Some preclinical models rely on pharmacology. The dopaminergic model of schizophrenia is based on the efficacy of D2 receptor antagonists for treating hallucinations and delusions. Likewise, psychosis and schizophrenia-like cognitive deficits induced by treatment with N-methyl-D-aspartate (NMDA) glutamate receptor antagonists such as phencyclidine (PCP) and ketamine have given rise to the glutamate hypothesis of schizophrenia. Some models of schizophrenia therefore make use of the treatment of animals with these drugs. Genetic models also try to incorporate these hypotheses such as the knocking out of NMDA glutamate receptor subunit NR1 subunit which induces a set of schizophrenia-like symptoms [8]. Regardless, models dependant on one risk factor rarely replicate symptoms all-encompassing of the disease, nor are they expected to [7].

While the aforementioned models are crucial to our understanding of schizophrenia, they do not paint a complete picture of the disorder. Monozygotic twins who share exactly the same DNA do not also deterministically share the same neuropsychiatric condition – heritability is 80% for schizophrenia [9]. Consequentially, research in this field must consider the interplay between genetic and environmental factors [10].

Association between immunity and schizophrenia

Among the diverse set of risk factors, dysregulation of the immune system dependably stands out as a player in the pathophysiology of schizophrenia [11]. Properly tuned, the immune system promotes inflammation in response to pathogens, cell damage due to infection, aging, disease, and irritants. Inflammation is orchestrated by a variety of soluble molecules such as cytokines, chemokines and acute phase proteins [12]. By recruiting the action of specialized cell populations, inflammation can destroy pathogens, remove unhealthy cells, and aid cell recovery. Pro-inflammatory cytokines also activate the production of reactive oxygen and reactive nitrogen species (ROS and RNS) which are toxic to invaders [13]. When dysregulated, inflammation can cause cell injury and death [13].

Early evidence to implicate the immune system in schizophrenia was the observation of an increased prevalence of elevated serum levels of antibodies against nuclear proteins [14], brain proteins (including anti-amygadala, prefrontal cortex, and cingulate gyrus) [15], nicotinic acetylcholine receptors (nAChR) [16] and heat shock proteins (HSPs) [17]. Additionally, increased levels of cytokines interleukins-1β (IL-1β) and IL-6, and cytokine receptor IL-2R [18,19] in schizophrenic patients gave credence to the hypothesis that chronic activation of macrophages and T lymphocytes help mediate the pathophysiology of schizophrenia along with their secreted cytokines: IL-1, IL-2, tumor necrosis factor (TNF), and interferon IFN-α and -γ [20]. For example, IL-2 can mediate neurotransmission, and especially dopamine activity [21]. Other cytokines have been found to affect serotonin, dopamine and glutamate neurotransmission and even lead to

morphological changes in the brain relevant to neuropsychiatric disorders [22–26]. The immune reaction against infections was beginning to garner attention as a player in the pathophysiology of schizophrenia.

Association between maternal infection and neuropsychiatric disorders

The potential link between immunity and schizophrenia directed interest toward infection. Karl A. Menninger reported an association between prenatal influenza exposure during the 1918 pandemic and later development of psychotic disease by following 80 patients, 25 of which were diagnosed with dementia praecox, at the Boston Psychopathic Hospital [27]. Only after better understanding of dementia praecox led to a new diagnosis and was replaced with schizophrenia, did Fuller Torrey bring back the immune hypothesis of psychotic disorders in 1970 linking latent viruses to schizophrenia [28]. In the next decade, Mednick associated increased risk of schizophrenia to prenatal exposure during the Helsinki influenza epidemic [29] lending increased interest to the role of the immune system in schizophrenia. Epidemiological studies also associated immune stress during gestation with autism spectrum disorder (ASD) [30,31], bipolar disorder [32], and cerebral palsy [33]. Since then, epidemiological studies have revealed other infectious agents that play an etiological role in schizophrenia, autism, related disorders and mood disorders [34,35]. For example, other viral agents include rubella [36], measles [37], polio [38], herpes simplex [39]. Beyond viral agents, other types of infections are considered risk factors for schizophrenia such as sinusitis, tonsillitis and pneumonia caused by bacteria [40], genital and reproductive infections [41], and hosting the protozoan parasite *Toxoplasma gondii* [42]. Postmortem studies strengthened the correlation [43,44]. Clinical examination and serological testing reports show a 10- to 20-fold increased risk of

developing schizophrenia after prenatal exposure to rubella [36]; 7-fold risk after exposure to influenza in the first trimester; 3-fold risk after infection in early to mid-gestation [41]; and 2.5-fold if maternal antibodies against Toxoplasma gondii are present [45,46]. Meanwhile, some studies disagree. For example, Buka et al. [47], could not confirm the role of herpes simplex virus reported by Brown et al [48]. While both epidemiological and experimental evidence suggests that immune stress at any of the prenatal, perinatal and early postnatal periods imparts risk, earlier environmental insult may have a greater impact because affecting early proliferation and differentiation may go on to perturb the ability of some cells to migrate, select target, or mature [49,50]. Visibly, maternal immune activation due to infection presents itself as an important risk factor that provides additional variability of risk to heritability.

While gestational immune stress is of particular interest, it is one dimension of a broader hypothesis that stress during gestation has damaging effects on fetal brain development which may later manifest as a variety of mental illnesses: psychotic, developmental or mood [49]. Beyond any specific pathogen, fevers were associated with autism [24,51,52]; especially the severe ones that were diagnosed in hospitals rather than outpatient settings [53]. Autoimmune disorders, allergies, asthma, acute stress, and exposure to pollutants are all risk factors [54]. Despite their complexity, these disorders share a few symptoms and pathophysiological traits and comorbidities are often observed [55]. For example, prenatal infections linked to schizophrenia were also correlated with low scores on the Winconsin Card Sorting Test and Trails B which measure cognitive ability that is relevant to multiple neuropsychiatric disorders [45,56]. Immune stress during fetal development is correlated with wide range of symptoms.

Immunity during Pregnancy

Pregnancy modulates the immune system to optimize maternal protection and fetal tolerance [57]. During pregnancy, the endometrium or uterine lining swells to become the decidua, and together with invading fetal trophoblasts, becomes the placenta, the interface between mother and fetus [58]. The fetal syntiotrophoblasts of the placenta are well-equipped to deal with mediators of the immune system [59]. For example, they do not express major histocompatibility complex (MHC) class II and will not be recognized by Cluster of Differentiation CD4+ cells [60]. The fetus is also uniquely protected from the immune system because trophoblast cells do not express the MHC genes (HLA-A and B) that are responsible for allograft rejection in humans. In this way, MHC presented on the membranes of trophoblasts promote tolerance [61]. However, the fetus is not prepared to balance the excessive infiltration of immune mediators caused by great immune stress [49,62].

A specialized natural killer cell (NKC) population, uterine NKCs, makes up 70% of the leukocytes during the first trimester and confer cytotoxic immunity by killing appropriate cells via antibodies and apoptosis [63]. A shift away from cell-mediated immunity is central to this effect [59]. The adaptive immune system consists largely of leukocytes (T and B cells) [64]. B cells confer humoral immunity [64]. T cells may have CD4 or CD8 on their cellular membrane [65]. CD8+ T cells confer protection via cytotoxic mechanisms [65]. Once activated, CD4+ T cells will become helper T cells (either Th1 or Th2) [66]. Th1 cells provide cell-mediated immunity while Th2 cells promote protection against pathogens outside cells such as parasites and allergens and are vaguely anti-inflammatory [66]. Pregnancy is generally marked with an immunosuppressant shift from Th1 to Th2 immunity [67].

Additionally, T cells with CD4 and CD25 belong to the regulatory T cell group. They also play a protective role against rejection of the fetus. They suppress activity of Th1 and Th17, another regulatory helper T cell type [57]. This shift is regulated by signals from the placenta and hormones such as estradiol, progesterone, and human gonadotropin [68,69]. The modulation of gestating mothers' immune system change their response to pathogens, often making them more susceptible to infection [70]. Improvement or exacerbation of autoimmune disorders may be seen depending on their dependency of cell-mediated immunity [71].

In mice, overstimulation of the immune system of pregnant dams increases pro-inflammatory cytokines in blood and amniotic fluid [72] and in humans, small amounts of cytokines have been shown to cross placenta [73]. The blood-brain barrier of human fetus is also more permeable and allows cytokines through which can trigger more cytokine signaling in the brain [74]. Offspring later develop an enhanced proinflammatory immune response, favoring Th1 and Th17 cells activation [75].

Morelli et al. [57] analyzed DNA from the Autism Genetic Resource Exchange database and found a greater occurrence of polymorphisms in proinflammatory cytokine genes in mothers of children with ASD. They interpret that this means mothers of autistic children have the genetic inclination to respond more strongly to immune stress. They also show the children of these mothers inherit a readiness to respond to infection [57]. These children of mothers with more stubborn pro-inflammatory cytokines genes fit the theory that sensitivity of the immune system plays a role in the development of neuropsychiatric disorders.

Fetal development is a vulnerable period for immune insult. The healthy development of the fetus is dependant on the precise balance of cytokines between the maternal and fetal environments separated by the placenta [76,77]. Cytokines are small proteins secreted for cell signaling with both local and global effects. During the innate immune response, they mediate growth, maturation and response of immune cells to balance between humoral and cellular immune responses [76]. Yet, cytokines also participate in the regular communication between cells of other tissues. In the nervous system, cytokines are secreted during development to mediate neural lineage restriction and commitment, progenitor cell proliferation and survival, and neuronal differentiation [12]. IL-6 regulates the self-renewal of neuroepithelial and radial glia cells (RGC), the scaffolds and precursors for migrating neurons and then shift neurogenesis to gliogenesis [78]. This cytokine also inhibits neurogenesis from the subgranular zone [77]. Later, cytokines, specifically IL-6 and IL-1b mediate axodendritic process outgrowth. Chemokines, a type of cytokine with chemoattractant properties can form gradients that guide axon paths [77]. Cytokines even affect neurotransmission, enzymes and synaptic organization [76].

Brown et al. [56] determined specific cytokines present during gestation associated with schizophrenia. They found significantly elevated IL-8 levels during the second-trimester in mothers of offspring with schizophrenia spectrum disorders compared to the mothers of comparison subjects. Meanwhile, there was no comparative difference in levels of the other major pro-inflammatory cytokines, IL-1β, IL-6, or TNF-α [56]. Buka et al. [39] compared cytokine levels in the third trimester. They found significantly higher levels of TNF-α but not the other interleukins IL-1b, IL-6 [39]. Garay et al. [79] reviewed MIA effects on cytokine levels by systematically studying the region- and age-specific cytokine alterations of MIA. Overall, they discovered MIA has long

term effects on a wide range of cytokines; they are altered differently in different regions and at different ages, giving MIA the potential to affect multiple development processes through different inflammatory pathways [79].

MIA as a model

While epidemiological studies with large human population samples have gathered critical evidence of a correlation between prenatal infection and eventual development of neuropsychiatric disorder, testing causality of this risk factor cannot be studied in humans for ethical reasons. To delve into the detailed cellular and molecular mechanisms of the pathophysiology of these disorders and prepare novel treatments, animal models are the key [49].

Pioneers such as Zuckerman et al. [80] experimented with perinatal immune activation until Fatemi et al. [81] established an experimental model where mice were nasally exposed to a mouse-adapted human influenza virus strain at various gestational days. Different immunogens have been used since. Activation of the immune system during gestation has been named the maternal immune activation (MIA) model and has been extensively reviewed [24,35,49,82,83]. The joined literature speaks to the causal relationship between gestational immune activation and deficits relevant to the study of neuropsychiatric disorders in experimental mice models. Such deficits comprise impaired development of the cortex and corpus callosum, pyramidal cell atrophy, reduced hippocampal volume, loss of gamma-aminobutyric acid (GABA) interneurons, and reduced serotonin levels [35,81,84–87]. These neurophysiological insults are accompanied by behaviour deficits after puberty.

These include impaired sensorimotor gating [88], attentional control of associative learning [89], working memory [90], executive function [91], social behaviour [92], glutamate-associated neurotransmission [93], increased stereotypy [92] and dopamine associated neurotransmission [94,95]. Some of these behavioural abnormalities can be ameliorated with both typical and atypical drugs such as chlorpromazine and chlozapine [35,41,82,86]. Meanwhile, the mother's behaviour is unaltered [96]. The MIA model has been established as an appropriate model to study the causal effects of fetal immune stress on the development of animal conditions relevant to human neuropsychiatric disorders.

MIA pathways

While the physiological and molecular events connecting gestational immune activation and the fetus' eventual development of mental illness are unknown, more is understood about MIA pathways in the animal model framework. MIA models in animals require introducing pathogen-associated molecular patterns (PAMPs) to be recognized by pathogen recognition receptors (PRRs) [24]. As part of the innate immune system, PRRs recognize specific molecules associated with intrusive and dangerous pathogens, PAMPs, and others associated with cell damage, damage-associated molecular patterns (DAMPs) [97]. MIA is sometimes modeled by injection of the human influenza virus which is comprised of multiple PAMPs throughout its cycle in the body [98]. Multiple PPRs will therefore be recruited to activate diverse pathways in a MIA model using an influenza virus as an immunogen. For example Toll-like Receptors (TLR) are a non-catalytic type of PRR that recruit cytosolic adapter proteins once activated [97]. Endosomal membrane receptor TLR-3 that recognize double-stranded RNA, may also be activated by a by-product of digested phagocytized virus-infected cells in dendritic cells and B lymphocytes [99,100]. TLR-7 and 8 recognize viral single

stranded RNA [101]. Together, TLRs activate transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and activator protein 1 (AP-1) via a TIR-domain-containing adapter-inducing interferon-β (TRIF)-dependent pathway to induce release of pro-inflammatory cytokines such as interleukins IL-1b, IL-6, IL-8 and tumor necrosis factor TNF-α [100]. The TRIF dependent pathway also leads to apoptosis and release of anti-viral interferons by interferon regulatory factor (IRF)-3 activation via a mitogen-activated protein kinase (MAPK)-dependent pathway [100]. Rig-1 recognize 5' triphosphate RNA in the cytosol [102]. Also in the cytosol, NOD-like receptor family member NOD-, LRR- and pyrin domain-containing 3 (NLRP3) is a multiprotein inflammasome complex which is recruited by the condition of a few factors during infection with the human influenza virus [102]. Clearly, influenza virus triggers an innate immune response at many sites.

Other models of MIA rely on PAMPs that have a more precise target. Polyinosinic-polycytidylic acid (PolyI:C), a synthetic double-stranded RNA composed of inosine and cytidine, either organized randomly or as inosine strands hybridized with cytidine strands, is recognized by TLR-3. Lipopolysaccharides (LPS), a bacterial endotoxin, is recognized by membrane receptors TLR-4 of granulocytes, B-lymphocytes and monocytes and engages TRIF-dependent and myeloid differentiation primary response gene 88 (MyD88)-dependent pathways to activate NF-kB and AP-1, also leading to the release of pro-inflammatory cytokines. Injecting immunogens such as poly(I:C) or LPS allow more precision than injecting influenza virus, with immune activation lasting 1 to 2 days only [35,103].

These MIA animal models all seem to rely on the secretion of IL-6 since IL-6 knock out (KO) models are protected from prenatal immune activation. Meanwhile, evidence shows human patients diagnosed with schizophrenia and autism have altered blood levels of cytokine, especially pro-inflammatory IL-6 [104,105]. IL-6 activates Th17 which maintain mucosal barriers. In disease, Th17 have been linked to depression. Here, they have been recognized as an important downstream element of the MIA paradigm. MIA causes alteration of IL-17 signalling, a major cytokine secreted by th17 [106]. Choi et al. [106] determined that genetically knocking out RORγt-dependent effector T lymphocytes such as Th17 or blocking the effector cytokine IL-17a with antibodies ameliorated ASD-relevant behaviours – ultrasonic vocalization, sociability and repetitive behaviour – in MIA mice prenatally treated with poly(I:C) on embryonic day E12.5. Injecting any of IL-6 or IL-17, is enough to induce MIA-induced phenotypes [54,106,107]. Enhancing anti-inflammatory IL-10 cytokine expression genetically can normalize behavioural abnormalities in MIA model mice in terms of sensorimotor gating, anxiety and locomotion [108]. Despite understanding that proinflammatory cytokines must play a role in the etiology of MIA-induced neuropsychiatric conditions, little is understood about the mechanism by which these effects are manifested.

Yet, many processes are susceptible to be affected depending on the timing. For example, immunogen injections performed E9-E10 coincide with the conversion of neuroepithelial cells into radial glial cells and the onset of neurogenesis [77,109]. It is also known that cytokines can have many effects on the developing brain. For example, IL-1B can inhibit cell proliferation and neurogenesis [110]. In the literature, MIA models for ASD activate maternal immune system later, while schizophrenia models favor E9.5 [111]. E9.5 coincides with formation of the neural tube in mice and is also when microglia start colonizing the brain [112]. Schizophrenia related symptoms such as

sensorimotor gating deficits are generally observed when prenatal injections of immunogens are performed around E9.5 or E12.5 whereas E16 and E17 injections manifest increased seizure susceptibility, reduced social skills, and cognitive deficits, symptoms more closely related to ASD. [49,113,114].

While both immune and stress models of gestational environment produce elevated corticosterone levels and both IL-6 and corticosterone can cross the placenta and blood-brain barrier, double hit experiments where both MIA and juvenile stress were performed did not demonstrate interaction [115–119]. Yet, other fruitful studies make use of double-hit models, pairing the MIA model with genetic models of SZ or ASD such as DISC1 and α7 nAChR [83,120–122] and NRG1 (which encodes Neuregulin-1) [123], NR4A2 (for Nurr1) and TSC2 which all synergize to create greater phenotypes than independently. [54] MIA models, despite their diversity, improve our understanding of the environmental risk factors of primarily genetic mental illnesses.

MIA Translational power

The maternal immune activation has been broadly studied using methods implicating diverse dose sizes, strains, and developmental ages [35,118]. Yet, there is no consensus on a standard of methodology for this model and it is therefore difficult to compare different results. It is important to consider the validity of this model [50]. Validity is evaluated in three parts. Construct validity requires a model to involve a known risk factor of the disease being modeled. Secondly, a model with good face validity will induce phenotypes comparable to known signs and symptoms of the disease. Finally, predictive validity refers to the ability of the model to predict treatment outcomes

from model to human disease. It is estimated by testing if known treatment methods for the disease are also efficient in the model [83].

The maternal immune activation model of neuropsychiatric disorders has construct validity because of the evidence supporting correlation between schizophrenia and autism cases and immune activation during gestational period [49,95]. Though the model has construct validity, it is a single-risk model and can therefore not be expected to recreate the whole disease. Another issue with animal models of neuropsychiatric disorder arise from the fact that these disorders, as far as they are genetic disorders, do not arise from one gene mutation but a various combinations of numerous gene mutations and how they interact in an environment of human cells and human proteins [10].

Face validity is also adequate because of the numerous behavioural and structural deficits present in the MIA model which are relevant to neuropsychiatric disorders [50]. A challenge in creating valid animal models is the heterogeneity of neuropsychiatric disorders. Two individuals with the same diagnosis may have little symptoms in common (for DSM diagnosis, they need only express a specific number of symptoms from a list). Meanwhile multiple disorders share some symptoms. Additionally, many of these symptoms cannot be measured objectively (ex. hallucinations, delusions, specific emotions) which are subjective experiences that can only be communicated by language). Abnormal social behavior, motivation, working memory, emotion, and executive function can be measured in animal models but the extent of their respective translational power must be kept in mind. [124]

The principal behaviour test for studying schizophrenia-like symptoms in rodents is the prepulse inhibition (PPI) to acoustic startle response (ASR) test. This measures sensorimotor gating, an ability to filter sensory information and respond to most salient information. MIA mice generally show impaired PPI, thus supporting construct validity of the model [35,108]. This phenotype is associated with positive symptoms and dopaminergic neurotransmission regulation. Amphetamine-induced hyperlocomotion is also present in MIA mice [125]. This is the second test to evaluate positive symptoms in the absence of tests for hallucinations and delusions. They also show stereotypic or repetitive behaviours [126]. Offspring of the rodent MIA model have enlarged ventricles, are more sensitive to amphetamine and hallucinogens, display changes in dopamine and serotonin neurotransmission, increased anxiety, reduced sensorimotor gating, and presence of stereotypic behaviour [111,127]

Social interaction deficits in MIA rodents can be observed as early as in pup isolation ultrasonic vocalizations [83]. In adults, sociability is measured by letting a mouse choose between the area with another mouse subject or without in a three or one chamber box. Anxiety can be tested in open field (OF) test, elevated plus maze (EPM) test or light-dark box test. Most studies find prenatal treatment with immunogens increases levels of anxiety in MIA offspring as measured in OF, EPM and foot shock tests (FST) and stress-induced corticosterone levels [119,128–131]. Meanwhile, Chlodzinska's lab found prenatal LPS was anxiogenic when anxiety was tested with EPM and anxiolytic when tested on OF [132]. Others have found anxiety on EPM was also decreased [133]. Anxiety reduction was also reported in light-dark box, EPM, and FST [134]. Finally, other studies have found no significant difference in anxiety in their MIA model [118,135,136]. Another study

determined sex-difference; male offspring but not females experienced increased anxiety and depression in their poly I:C MIA model [137].

Finally, predictive validity has been confirmed. The efficacy of known antipsychotic on MIA makes it more probable that interventions that work in rodent MIA models will be worth exploring to translate into treatments for psychotic illnesses [138].

MIA model in rhesus macaque monkeys also show repetitive behaviors, abnormal communication and impaired social interactions that have early onset and intensify as they get older [54,139]. Replicating MIA-induced behaviour deficits in other species allow to predict translation ability. If the pertinent mechanisms were conserved during evolution, they may have been conserved in humans [140]. Yet, mice and rats are low cost, have short gestational period, are less ethically problematic, and have many genetic modifications available. Studies in rats are better suited to explore the effects on communication [141]. Monkeys have more complex brains and make use of a wider variety of social behaviours, including facial expressions and gestures as well as vocalization but are more ethically problematic [83,142]. Together, multiple species have shown MIA model to be a valid model of environmental risk for neuropsychiatric disorders. The MIA model can act as a useful tool to study the effects of changes in activity of candidate proteins relevant to these disorders.

Protein tyrosine phosphatase 1B

Protein tyrosine phosphatase PTP1B has been proposed as a protein that may potentially help regulate the inflammatory effect of the MIA model because of its role in inflammation and increased activity of upregulating gene YBX1 (encoding Y-box binding protein 1) in MIA [143]. Protein tyrosine phosphatase 1B (PTP1B) is a 435 amino-acids long non-receptor cytosolic tyrosine phosphatase predominantly localized in the endoplasmic reticulum [144]. PTP1B was the first tyrosine phosphatase to be discovered. Originally isolated from human placenta tissue [145], PTP1B is in fact a ubiquitous protein that can be found in most cell types [146]. Human PTP1B is encoded by the *ptpn1* gene. Once mature, it is composed of two essential functional regions. The first 35 residues of the C-terminal make up a hydrophobic stretch that targets and interacts with the endoplasmic reticulum thus regulating protein tyrosine phosphatase activity by limiting its access to substrates [144]. The catalytic region faces the cytoplasm. The mechanism by which PTP1B dephosphorylates tyrosine residues consists of two-steps. The first step is a nucleophilic substitution: the electron rich Cys215 residue selectively bonds the partially positively charged phosphate atom of the protein tyrosine phosphate, while Asp181 protonates the oxygen atom of tyrosine, forming a thiophosphate intermediate and a dephosphorylated protein tyrosine residue. In the second step, the catalytic intermediate is hydrolyzed with a water molecule guide by Gln262 while Asp191 acts as the general base. The phosphate is released and the enzyme recovers its original state [147].

Many cellular signaling events are regulated by proteins' level of tyrosine residue phosphorylation. PTP1B, in balance with other kinases and phosphatases, therefore play a regulatory role in a multitude of processes, including inflammation, cell-adhesion, metabolism, tumor formation and

mental disorder pathophysiology [147]. PTP1B binds both insulin receptors and their insulin receptor substrates IRS-1, in non-neural and neural cells alike, removing phosphate from tyrosine, thus deactivating the insulin receptor cascade and providing insulin resistance [148].

The role of PTP1B in the crosstalk between metabolism and immunity has been studied as insulin resistance was associated with adipose tissue inflammation. During inflammation, Tumor necrosis factor (TNF) increased PTP1B activity via the IKK/NF-kB pathway leading to insulin resistance [149]. PTP1B is sensitive to the oxidative stress that accompanies inflammation. PTP1B can be regulated by the level of serine/threonine phosphorylation, calpain-cleaving, and oxidation [147]. Countering the oxidation of PTP1B by tobacco smoke with glutathione peroxidase (GPx-1) was found to disrupt modulation of inflammation in chronic obstructive pulmonary disease (COPD) and block release of cytokine IL-17 [150]. Oxidative stress has been observed in the hippocampus following prenatal LPS treatment [151]. Treatment with molecular hydrogen ameliorated working memory deficit neuron and oligodendrocyte loss in the cortex and amygdala in a MIA model [152].

While research on PTP1B's role in illness is more pronounced in the fields of diabetes and cancer, roles in neuropsychiatric disorders have been established. MECP2 is an X-linked gene for methyl-CpG-binding protein 2 (MeCP2), a transcriptional regulator which plays an important role in Rett syndrome and in cognitive disorders, autism, and early-onset schizophrenia [153]. MeCP2 mainly affects motor functioning, social interaction, cognition, and stereotypy [154]. MECP2 acts by suppressing PTP1B, thus enhancing tropomyosin receptor kinase B (TRKB) protein tyrosine

kinase response to brain derived neurotrophic factor (BDNF) [153]. PTP1B's role in Rett syndrome demonstrates the potential to affect neurological pathways relevant to neuropsychiatric disorders.

Another link has been made between PTP1B and mental illness. Calcium/calmodulin dependant kinase 2 alpha, Camk2α-Cre conditional KO of mice has allowed to demonstrate LMO4 acts as an inhibitor of PTP1B to maintain leptin and insulin signaling in the hypothalamus [155]. It also has proved to be important in cognitive function in the hippocampus, regulating calcium-induced calcium release and synaptic plasticity in the hippocampus [156]. LMO4 has been proposed as a candidate gene for schizophrenia based on genome-wide analysis [157]. Its altered expression in schizophrenic patients and its specific pattern of dysregulation could affect asymmetric perisylvian cortex development, an area which may later be involved in auditory hallucinations in schizophrenic patients [158]. Supporting LMO4's candidacy, Levchenko et al. [159] then reported an increased prevalence of LMO4 polymorphisms in schizophrenia patients.

A downregulation of LMO4 gene expression was reported in a MIA mouse model induced with influenza virus [160]; therefore, decreased inhibition of PTP1B could be an important signaling dysregulation that contributes to expression of some behavioural deficits in MIA models. Additionally, both expression and activity of PTP1B are increased by injection of IL-6 [161]. When IL-6 is injected prenatally, phenotypes observed with MIA model are replicated [107]. PTP1B regulatory gene YBX1 is also increased in MIA model and PTP1B has been hypothesised as a

protein that may help regulate the inflammatory effect of the MIA model [143]. Because activity of regulatory proteins of PTP1B is modulated in MIA, PTP1B dysregulation possibly occurs in MIA.

PTP1B's role in inflammation and mental disorders make it interesting to study in the context of MIA and neuropsychiatric disorders that arise from gestational immune stress. For example, a loss copy-number-variation mutation of the *ptpn1* gene has been associated to autism [162,163]. Additionally, our lab has demonstrated that metabotropic glutamate receptor mGluR5-dependant endocannabinoid signaling disruption in PTP1B inhibitor LMO4 knockout mice is anxiogenic [164]. The anxiolytic LMO4-PTP1B-metabotropic glutamate receptor 5-endocannabinoid cascade in the amygdala could be also be disrupted in MIA models and contribute to their anxiogenic effect. It is unknown whether PTP1B plays any role in the development of mental deficits of offspring affected by gestational immune stress.

Objectives

Rodent MIA models give rise to different behavioural phenotypes, which help study gestational immune activation in the development of neuropsychiatric disorders such as schizophrenia. Meanwhile, PTP1B shows to be an interesting candidate to play a role both in neuropsychiatric disorders such as schizophrenia and dysregulation of the immune system during fetal brain development. Therefore, this study aims to establish a rodent MIA model and evaluate its behavioural phenotypes. Then, we aim to knock out the PTP1B gene in camK2 neurons of control and MIA mice.

Hypothesis

We hypothesise prenatal treatment with poly(I:C) will lead to symptoms relevant to the study of schizophrenia in rodents, i.e., increased locomotion, increased anxiety, deficits in working memory, and prepulse inhibition to the acoustic startle response. Furthermore, we hypothesise that conditional knock out PTP1B in CamK2a neurons will protect against these MIA-induced behaviour deficits.

Materials and Methods

Animals

Male CamK2α$^{cre/-}$/PTP1B$^{flox/flox}$ (PTP1BKO) and female CamK2α$^{-/-}$ PTP1B$^{flox/flox}$ (WT) mice were bred on a C57BL6 background as described previously[165]. PTP1B KO females and WT males were not used for breeding to ensure all PTP1B KO are heterozygous. Animals were kept in a 12/12 hour day/night cycle. All animal use was approved by the University of Ottawa Animal Care and Veterinary Service and procedures were performed in accordance with Canadian Council on Animal Care guidelines.

MIA model

Female CamK2α$^{-/-}$ PTP1B$^{flox/flox}$ (WT) mice were bred with male CamK2α$^{cre/-}$/PTP1B$^{flox/flox}$ mice so that half of the progeny would be CamK2α$^{cre/-}$/PTP1B$^{flox/flox}$ (PTP1BKO) and half would be CamK2α$^{-/-}$ PTP1B$^{flox/flox}$ (WT). Gestational day 0.5 was identified as the day where vaginal plug was observed. Poly I:C potassium salt with buffer salts was obtained from Millipore Sigma and dissolved in milli-Q water. Dams were injected with 5 mg/kg poly I:C or saline vehicle intravenously through the right or left tail vein at gestational day 9.5. A battery of behaviour tests was carried out 3 months after birth: Beam Break test (BBK) on postnatal day 92, Open Field test (OF) on day 94, Elevated Plus Maze (EPM) on day 96, Y-maze (YM) on day 98, social interaction test (SI) on day 100 and prepulse inhibition PPI of the acoustic startle response on day 102. Before behaviour tests, mice were acclimatized for 30 minutes. All equipment was cleaned between each animal.

Beam break test

For this test, we used cages with fresh bedding placed inside a frame equipped with Micromax infrared sensors. Mice acclimatized to the room for 30 minutes**,** but the test started as soon as the mice were placed in their individual cage. Beam breaks were counted every minute for 2 hours. For our results, we used sequential beam breaks, also defined as ambulatory horizontal movement.

Open field test

Mice were tracked 10 minutes with Noldus Ethovision software in a 45cm x 45cm x 45 opaque, white box under 300 lux illumination. Mouse all began in the same corner of their box. The open center zone was defined as a 24cm x 24cm square in the middle of the box.

Elevated plus maze

Mice were tracked 10 minutes with an overhead camera using Noldus Ethovision software in an elevated plus maze under 100 lux illumination. The maze consists of four arms, two flanked with 20cm high black walls and the other two open. The arms are white. They are 6 cm wide and 75 cm long. They rest 74 cm off the ground. Mice began trial in center of the maze.

Y maze test

Mice were tracked 8 minutes with Noldus Ethovision software in a Y maze under 75 lux. The maze's three walled arms cross at equal angles. Each arm is 38cm long, 8cm wide and 13 cm high. Test started as soon as mouse was placed in the center of the maze; there was no acclimatization to the maze. All four limbs had to enter the arm zone to count as an arm entry. To calculate percentage alternations, the number of alternations is divided by the number of arm entries minus 2 and then multiplied by a 100%.

Prepulse Inhibition (PPI) of the acoustic startle response (ASR)

In the acoustic startle response PPI test, the mouse is loosely constrained in a sound cage with constant 65 dB background noise. A weak auditory stimulus 2-30 dB above background (prepulse) inhibits the response to a very loud 120 dB tone presented within 100 msec. The startle response is recorded on a pressure responsive platform and converted to an electrical signal measured in Volts. The prepulse inhibition is calculated as the percentage of the startle response to the pulse following a prepulse relative to the response to the pulse alone without the prepulse.

Statistical Analysis

Significant difference for all two-groups comparisons was analyzed using Student t-Test performed with Excel. Significant difference was defined by a p-value smaller than 0.05. Descriptive statistics were first assessed using Excel Analysis ToolPak to determine variance of the groups. If the variance for the group with bigger variance was no less than 3 times the variance of the group with the smallest variance, a homoscedastic Student t-Test was performed. Otherwise, we used a two-sample equal variance Student t-test. Males and females were separated for statistical analysis because MIA models often demonstrate sex-differences and number of animals for groups were unbalanced and female groups were relatively small.

For beam break results, multiple timepoints were being compared from two groups, therefore, two-way ANOVA was performed on R software. Factors were timepoint and group. Because samples were unbalanced, we used the type-III sums of square method. Because factors did not interact, the additive method was used last. The homogeneity of variance assumption was tested using Levene's test. If the p-value was no less than 0.05, the assumption was interpreted true. The normality assumption was tested using Shapiro-Wilk normality test. If the p-value was no less than 0.05, the assumption was interpreted true. If the two-way ANOVA determined that

group was a significant factor, a Student t-Test was performed between the total number of beam breaks for each group.

For prepulse inhibition results, inhibition was compared for three different prepulses, therefore, two-way ANOVA was also performed on R software as for the beam break results using the same tests to verify the homogeneity of variance and normality assumptions. Factors were defined as prepulse size and group. If the two-way ANOVA determined that group was a significant factor, a Student t-Test was performed between the two groups for each prepulse to determine for which prepulse group was a significant factor.

Results

MIA model

To evaluate the phenotypic expression of our lab's MIA model, we ran a battery of relevant behavior tests on 3 months old WT offspring. MIA male progeny mice exhibit increased locomotion, increased sensorimotor gating and reduced anxiety, but no changes in working memory while females only have reduced sensory gating compared to control mice. See (Table 1) for a summary of results.

A beam break test revealed adult WT male offspring of poly I:C treated dams demonstrated increased horizontal ambulatory locomotor activity compared to controls (Figure 1A). Females did not show effects in any group (Figure 2A) but their control score was relatively high in ambulation compared to males.

To evaluate the anxiety status of our MIA mice, we used the open field test and the elevated plus maze test. From prior studies, we expected MIA offspring to be more anxious[128,129,130,131,134]. Instead, in our hands, MIA produced the opposite effect. In the open field test, male offspring of poly I:C treated dams trended toward spending more time in the center zone and had more entries to the center (Figure 1B). This anxiolytic effect of our MIA model was supported by our elevated plus maze experiment. Offspring of poly I:C treated dams spent significantly more time than controls in the open arms but did not enter more often (open arm/closed arm ratio) (Figure 1C). Others have also reported an anxiolytic effect in MIA models[133,166,132,167]. The MIA model did not affect female mice's anxiety-like behavior (Figure 2B, C).

Despite increased locomotion observed in MIA mice in the 2 hour beam break test, no effects were observed due to prenatal treatment with poly I:C in distance travelled in the open field test or the number of entries onto open arms in the elevated plus maze (Suppl. Figure 3). These tests were done in an environment that tests anxiety and lasted 10 minutes. MIA effects in the OF and EPM paradigms are therefore more probably due to alterations of anxiety pathways than due to changes in locomotion or exploration.

Our MIA model did not exhibit an alteration in working memory, as assessed by the Y-maze test (Figure 1D, 2D).

Finally, we tested sensory gating with prepulse inhibition (PPI) of the acoustic startle response, a test of sensorimotor gating widely used for translational animal models of neuropsychiatric disorders. Impaired PPI is a hallmark of schizophrenia in patients[168] and mouse models[129] whereas both decreased[169] and increased[170] PPI have been observed in humans with autism. Increased[171,172,173,174], decreased[175] or unchanged[176] PPI have also been reported in mouse models of autism. Surprisingly, our male animals demonstrated a significant increase in PPI with prepulse 16 dB above background (Figure 1E), while female animals had decreased PPI for prepulses 8 and 16 dB above background noise (Figure 2E). No significant changes were observed to the acoustic startle response to the 120dB pulse due to prenatal treatment with poly I:C, indicating that the observed effects relate to prepulse inhibition and not baseline acoustic startle response (Suppl. Figure 4). Overall, our MIA model produces alterations in sensory gating, locomotion and anxiety in progeny mice in a sex-dependent manner.

Neuronal PTP1B KO effects on MIA model

To determine if PTP1B played a role in the phenotypes observed in our MIA model, we carried out the above tests neuronal PTP1B knock out mice. Interestingly, no effect was observed in KO male offspring of poly I:C treated dams compared to KO controls for ambulation (Figure 3A) indicating that PTP1B is required for the hyperlocomotor effect of MIA. There was no change in anxiety after prenatal treatment with poly I:C in PTP1B KO mice as measured by time spent in center and number of entries in the Open Field test (Figure 3B). The Elevated Plus Maze test showed similar results. MIA mice showed no anxiolytic effect in the PTP1B KO mice due to the prenatal poly(I:C) treatment on the time spent on open arms and number of entries (Figure 3C). Therefore, PTP1B is also required for the anxiolytic effect conferred by the MIA model. Similarly, when prepulse inhibition of the acoustic startle response was measured in MIA and vehicle control mice, MIA did not affect sensory gating of our PTP1B KO mice (Figure 3E). Female animals did not demonstrate significant difference in any of these tests (Suppl. Figure 1). Overall, MIA does not alter horizontal movement, anxiety, working memory or sensory gating in PTP1B KO male progeny.

Neuronal PTP1B knock out effects on mice

To ascertain that differences between PTP1B KO and WT mice in the MIA model were not due to differences between the mice's genotype, we compared the results for each test in mice treated with a vehicle containing saline solution at gestational day 9.5.

PTP1B KO male offspring of vehicle treated dams also demonstrated increased horizontal locomotor activity compared to their WT littermates for ambulation (Figure 4A). MIA induced

increased baseline locomotion is occluded in PTP1B KO mice, suggesting that inactivation of PTP1B in neurons might underlie the mechanism whereby MIA causes increased locomotion in WT male mice.

None of the other tests showed differences in behavior due to genotype (Figure 4B, C, D, E) indicating that the lack of behavior changes in PTP1B KO MIA mice is due to a protective effect of PTP1B ablation. Since MIA does not produce an anxiolytic effect in PTP1B KO mice as in the WT littermates, this could suggest that PTP1B ablation occludes the anxiolytic effect of MIA. It is worth noting that, PTP1B KO mice were not less anxious than WT littermates even under the basal conditions without MIA. In the case of female mice, no effects were observed from the conditional ablation of neuronal PTP1B gene (Suppl. Figure 2). Overall, neuronal knock out of PTP1B increases horizontal movement but has no effect on anxiety, working memory and sensory gating of male mice.

Discussion

Bathing in the nurturing environment conferred by the host mother's placenta, the fetus is at a crucial period of brain development. New neurons migrate to their appropriate positions and form the correct synaptic connections to create a functional neural network. If the mother is exposed to environmental stressors, inflammatory cytokines and corticosteroids can pass through the placenta and potentially affect these processes [177]. Activation of the immune system in gestating mothers has been identified as an important environmental risk factor for neuropsychiatric disorders such as schizophrenia and autism [24] as well as anxiety, depression and bipolar disorder [54] by charting historic outbreaks and epidemiological cohort studies. Maternal immune activation (MIA) animal models have been used to explore if and how the maternal immune system causes expression of pathophysiology in offspring by prenatal treatment with immunogens. MIA model studies have identified increase in locomotor activity [80,178], deficits in sensory gating from prepulse and latent inhibition experiments [129,80], deficits in working memory [113] and social interaction [139], anxiety-like [108], and depression-like behaviours [24,179]. While effects of MIA have been observed in the brain environment, neurological connectivity, microbiota, peripheral immune system, and behaviour [54], the specific pathways for these effects remain largely unknown. Most convincing evidence points at the elevation of pro-inflammatory cytokines in the developing fetal brain following MIA. PTP1B has been identified as candidate protein that could bridge inflammation to cognitive deficits in the MIA model [143].

Herein, we attempted to study the effects of immune activation during gestation on offspring adult behaviour by intravenous injection of 5mg/kg of polyI:C on E9.5. We described our lab's MIA model which exhibited sex-dependent effects in locomotion, anxiety, and sensory gating.

In accord with our expectations, our PTP1B KO mice were protected or occluded from all the changes induced by MIA. Our MIA mice fit the typical model where males were hyperactive, and females showed reduced sensory gating. Reduced PPI is generally the key phenotype of an MIA model useful for studying schizophrenia-related conditions in mice. A well calibrated MIA model should demonstrate this effect [111,127,180]. In our MIA model, only females show decreased PPI. Meanwhile, males demonstrate increased PPI. Foley's lab has previously shown increased PPI in MIA adult mice with lower prepulse while we observed increased PPI with higher prepulse [181].

Interestingly, a similar phenotype has been observed in a specific mutant model of Disc-1 gene where exon 2 and 3 are knocked out. Yamada and Kaibuchi's model manifests a deficit in PPI solely in females [182]. They also show sex-specific enhanced drug-induced locomotor hyperactivity in females. Both males and females have reduced anxiety and increased social ability in this model [183].

Generally, MIA has an anxiogenic effect on adult offspring [119,128–131]. Yet, the anxiolytic effect of MIA has been observed previously by Asiaei et al. [133] on the EPM test as they reported increased time spent on open arms and entries onto open arms while total arm entries remained unchanged for mice with prenatal exposure to LPS on E10. Solati et al. [166] replicated these results. As observed in our model, others have also found MIA effects on anxiety only in males but not females [137]. These paradoxical results have even been reported in the same mice. Chlordzinska et al. [132] found the anxiolytic effect with EPM and anxiogenic effect with OF. Our behaviour results are a reminder that the MIA model is complex and may have bidirectional

effects. The MIA model is sensitive to age, strain, and sex differences [184]**.** The lack of a standard procedure for the type of immune activator, the dose, and the gestational day of injection leads to a variety of phenotypic expression [118].

It is apparent that our mice react differently than expected to the MIA model. To better understand how results collected with these mice fit with the broader agglomeration of results in the scientific literature, tests should be conducted to see these gestating mother mice response to infection. Do they respond with increase cell-mediated immunity? Because these mice were not treated with a real virus, we would not have seen negative consequences of mothers not able to properly respond to a viral threat during pregnancy. Our specific mouse line might be more tolerant to immune stress. Our dams might have had a milder immune response to the polyI:C thus leading to fewer deficits. Alternatively, while pregnancy immunity commonly favors Th2 over Th1, the opposite is sometimes true. It would be interesting to see if our mouse line manifests a different pattern of immunity. This could explain unique offspring behavior phenotypes due to gestational exposure to immunogen.

Going forward, it would strengthen our model if we measured the effects of the model on cytokines levels such as IL-6, IL-1β, and TNF-α in both the mother and offspring. This was not done in the current study because these effects have been extensively studied in the past [133]. Given the uncommon phenotypes of our model, it would be interesting to know if we can observe a different immune reaction in our model compared to the more common models, especially in terms of pro-inflammatory cytokine levels.

Because PTP1B has demonstrated to play an interesting role, a crucial next step will be to determine how are PTP1B expression and activity affected in the MIA model, especially now that these effects don't seem in line with most of the literature. Yet, the expectations arose from inductive reasoning, since the literature did not inspect the specific changes of PTP1B or its endogenous inhibitor LMO4 specifically in neural cells but rather in all cells. While knowing whether PTP1B expression is increased or decreased following prenatal treatment with poly(I:C) does not tell the whole story, it would be helpful to determine if PTP1B expression and activity did in fact increase following treatment. If PTP1B demonstrates to be a potential target for gestational immune stress induced neuropsychiatric disorders, PTP1B inhibitor Trodusquemine could be used as a drug treatment in tests. Trodusquemine passes the blood brain barrier and is already in clinical trials for diabetes type 2 and obesity [185,186]. Trodusquemine will be useful in studying PTP1B's effect in mental disorders and if interesting,

To expand on this study which aimed to determine if PTP1B is a bridging protein between inflammation in MIA with development of cognitive deficits, it would be of interest to investigate the electrophysiological effects of MIA on neurons as well as the effects of PTP1B on these MIA-induced changes. With this goal, we performed a preliminary survey of electrophysiological changes due to PTP1B KO to identify a neuronal property that PTP1B may potentially be affecting in MIA.

It is interesting to note that the amygdala which plays a major role in anxiety, has been tied to locomotion. Chemical lesion of dopaminergic terminals in the BLA increases sensitivity to amphetamine-induced hyperlocomotion [187]. The amygdala participates in the locomotor

stimulating effect of the stress responsive hormone (CRH, corticotrophin releasing hormone) [188]. Optogenetic activation of a GABAergic pathway in the central medial amygdala projecting to the ventral-medial prefrontal cortex (PFC) was found to increase locomotion in the open-field test [189]. The basolateral amygdala (BLA) projects to the central medial amygdala [190]. Thus, hyperactivity of pyramidal neurons in the BLA of the PTP1B KO mice would be predicted to promote increased locomotion, as we have observed. Its role in anxiety and locomotion is why we focused our electrophysiological survey on pyramidal neurons of the basolateral amygdala.

In basolateral amygdala pyramidal neurons, postsynaptic but not presynaptic properties differ between WT and PTP1B KO mice under basal conditions. We next tested whether our genetic model affects presynaptic and postsynaptic properties of the basolateral amygdala pyramidal neurons. No effect was observed in train pulse experiments (Suppl. figure 5), suggesting no change in the presynaptic properties of the PTP1B KO neurons. There was a trend ($p=0.06$) toward reduced spontaneous excitatory postsynaptic currents (sEPSC) amplitude (but not frequency) (Suppl. figure 6A). sEPSC amplitude should be affected by the presynaptic residual calcium ions or the depletion of readily available vesicles. Changes in these tests are reflective of changes in release probability. Increased presynaptic release probability can also increase sEPSC frequency which was not observed. We observed no change in sEPSC amplitude either. Since experiments were performed with membrane voltage clamped at -70 mV, EPSC are predominantly AMPAR currents. Therefore sEPSC amplitude could be reflective of any postsynaptic change which regulates AMPAR and affect its permeability.

Importantly, excitability of basolateral amygdala pyramidal neurons was significantly increased in PTP1B KO mice (Suppl. figure 6B), suggesting that PTP1B is important to control neuronal excitability. PTP1B KO increased significantly increased firing rate of basolateral neurons (but not rheobase). The more excitable basolateral amygdala might communicate with central medial amygdala to confer anxiolytic effect [191] and then with the prefrontal cortex (PFC) to induce increased locomotion [189]. Membrane properties, including resting membrane potential, membrane resistance and capacitance, were not different in the BLA pyramidal neurons from vehicle-treated male progeny of PTP1B KO and WT littermates (Suppl. figure 7A, B, C). A key question that remains to be addressed is whether MIA causes increased BLA pyramidal neuron excitability in WT mice. If so, it would suggest that MIA inactivates PTP1B in BLA pyramidal neurons. Thus, unopposed tyrosine kinase activity would be associated with hyperexcitability of BLA pyramidal neurons and might account for increased locomotion. This instigates an interest for expending the present study by examining postsynaptic membrane excitability in WT and PTP1B KO progeny from control (vehicle) or MIA-treated dams. MIA-induced hyperlocomotor activity could be associated with increased excitability of BLA pyramidal neurons as observed in PTP1B KO.

In conclusion, we have contributed knowledge of a new pattern of behaviour changes (Table 1) induced by a MIA model which will be important moving forward as the scientific community standardizes the methodology to get models that better model the relevant human neuropsychiatric disorders they study, whether it be schizophrenia, autism, anxiety, etc. Secondly, we did not prove our hypothesis that PTP1B activity is increased by MIA. Conversely, our data suggests PTP1B is suppressed by MIA. We demonstrated an interaction between

neuronal PTP1B KO and our MIA model in terms of locomotor activity. As our study clarifies the paradigm of maternal immune activation whilst evaluating the effects of knocking out a regulatory protein, we are advancing the field in understanding how environmental factors may cause neuropsychiatric phenotypes.

Tables and Figures

Test	Phenotype measured	MIA WT		MIA PTP1B KO	
		Males	Females	Males	Females
Beam break	Horizontal movement	↑	=	=	=
Open field	Anxiety	↓	=	=	=
Elevated plus maze	Anxiety	↓	=	=	=
Y-maze	Working memory	=	=	=	=
Prepulse inhibition to acoustic startle response	Sensory gating	↑	↓	=	=

Table 1. Summary of treatment effects of prenatal polyI:C injections on behaviour phenotypes of 3-month old offspring and genotype-treatment interactions.

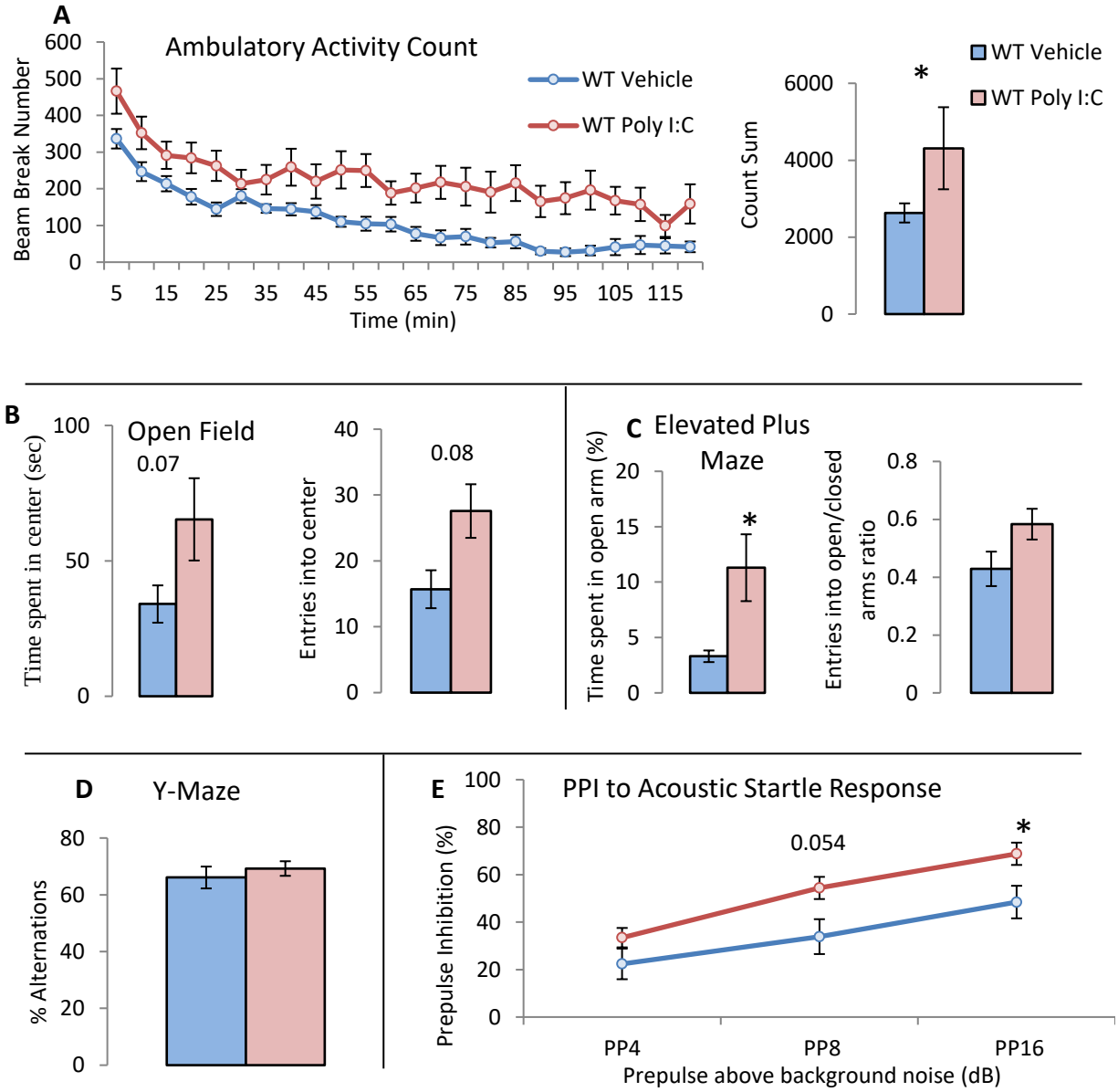


Figure 1. MIA model male mice have increased locomotion, reduced anxiety, a trend toward increased sensory gating but no changes in working memory.
A. Time course and sum of ambulatory (sequential) beam break number for WT males. B. Time and entries into open field center. C. Percent time in elevated plus maze open arms and entries into open/closed arms. D. Male percent alterations in Y-Maze. E. Prepulse inhibition for a prepulse 4, 8, and 16 dB above background noise. n=8, 12 WT male mice. Data are presented as mean ± SEM. *p<0.05; by student t-test.

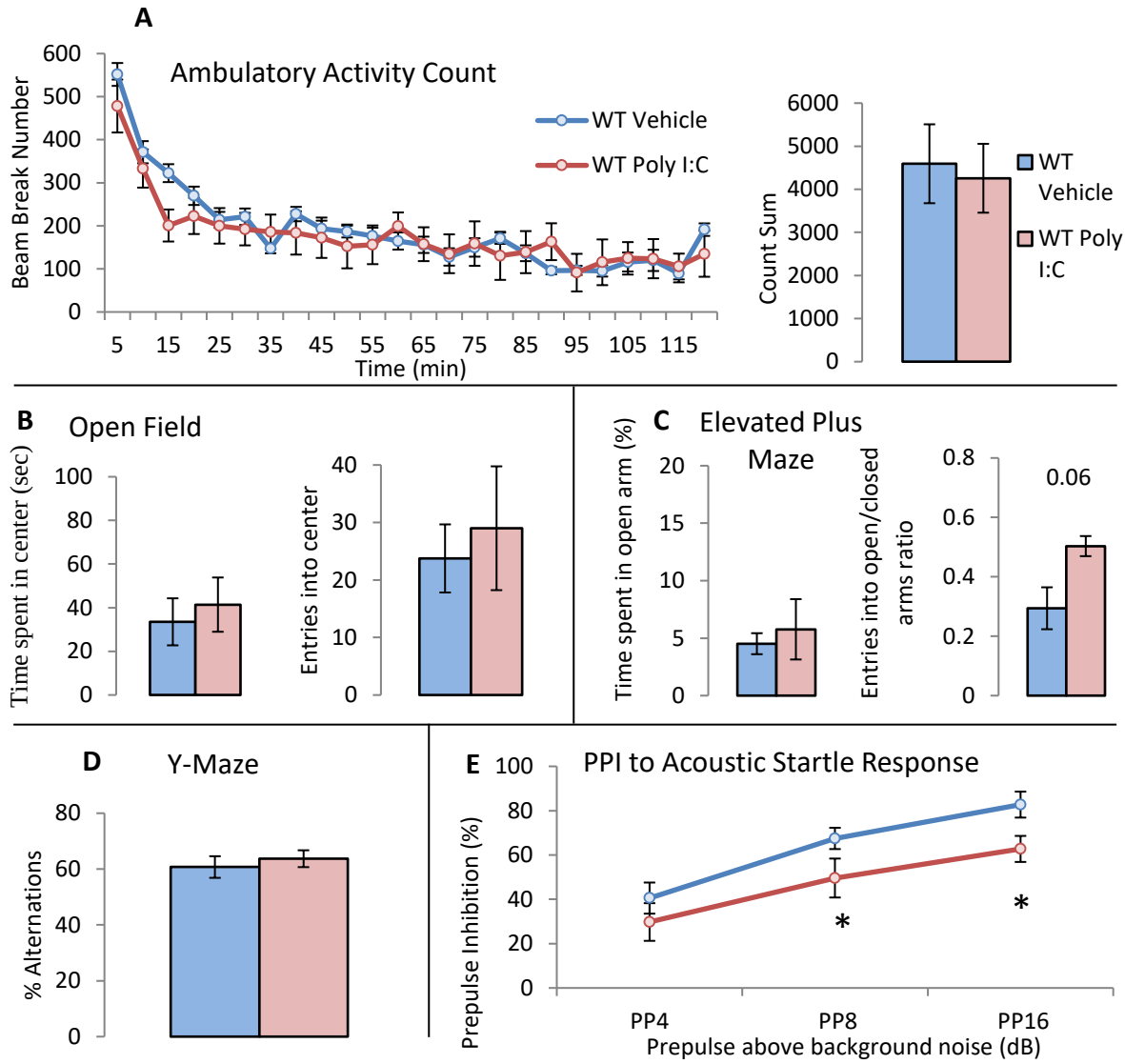


Figure 2. MIA model female mice demonstrate no changes in horizontal movement, anxiety, working memory but have reduced sensory gating.

A. Time course and sum of ambulatory (sequential) beam break number for WT females. B. Time and entries into open field center. C. Percent time in elevated plus maze open arms and entries into open/closed arms. D. female percent alterations in Y-Maze. E. Prepulse inhibition for a prepulse 4, 8, and 16 dB above background noise. n=4, 4 WT female mice. Data are presented as mean ± SEM. *p<0.05; by student t-test.

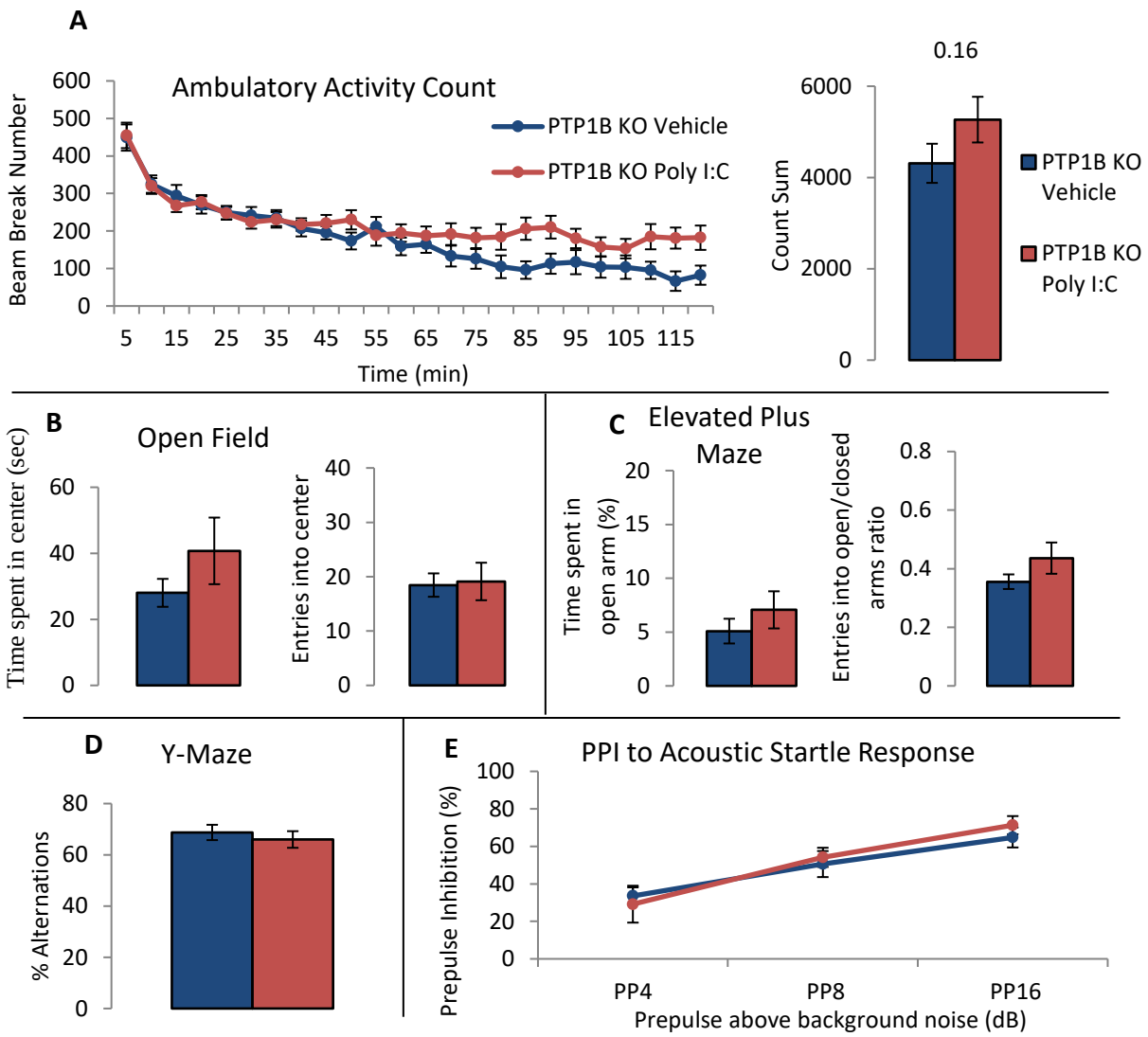


Figure 3. PTP1B KO male mice do not demonstrate any changes due to prenatal treatment with poly I:C in horizontal movement, anxiety, working memory and sensory gating.
A. Time course and sum of ambulatory (sequential) beam break number for PTP1B KO males. B. Time and entries into open field center. C. Percent time in elevated plus maze open arms and entries into open/closed arms. D. Male percent alterations in Y-Maze. E. Prepulse inhibition for a prepulse 4, 8, and 16 dB above background noise. n=16, 17 PTP1B KO male mice. Data are presented as mean ± SEM. *p<0.05; by student t-test.

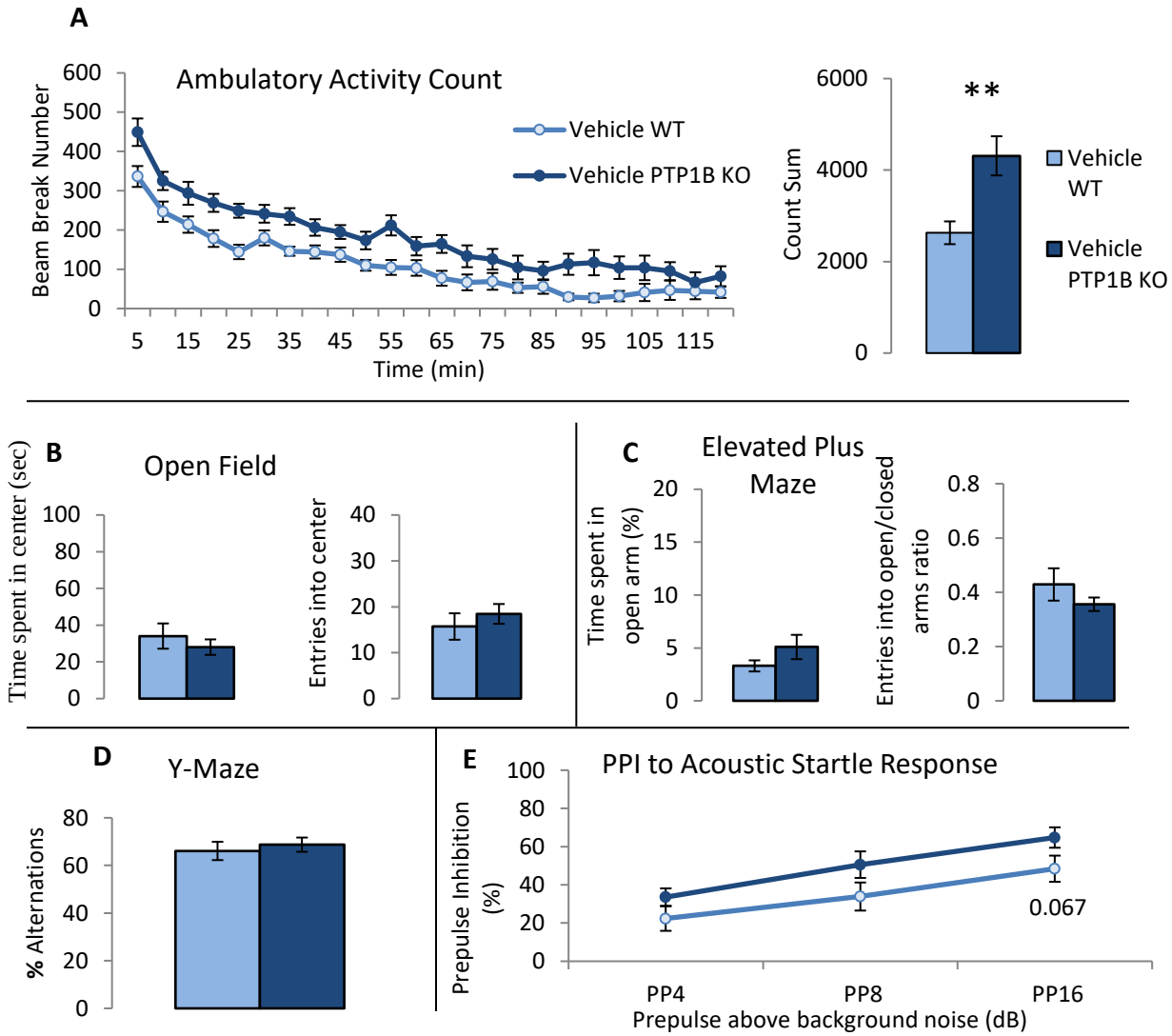
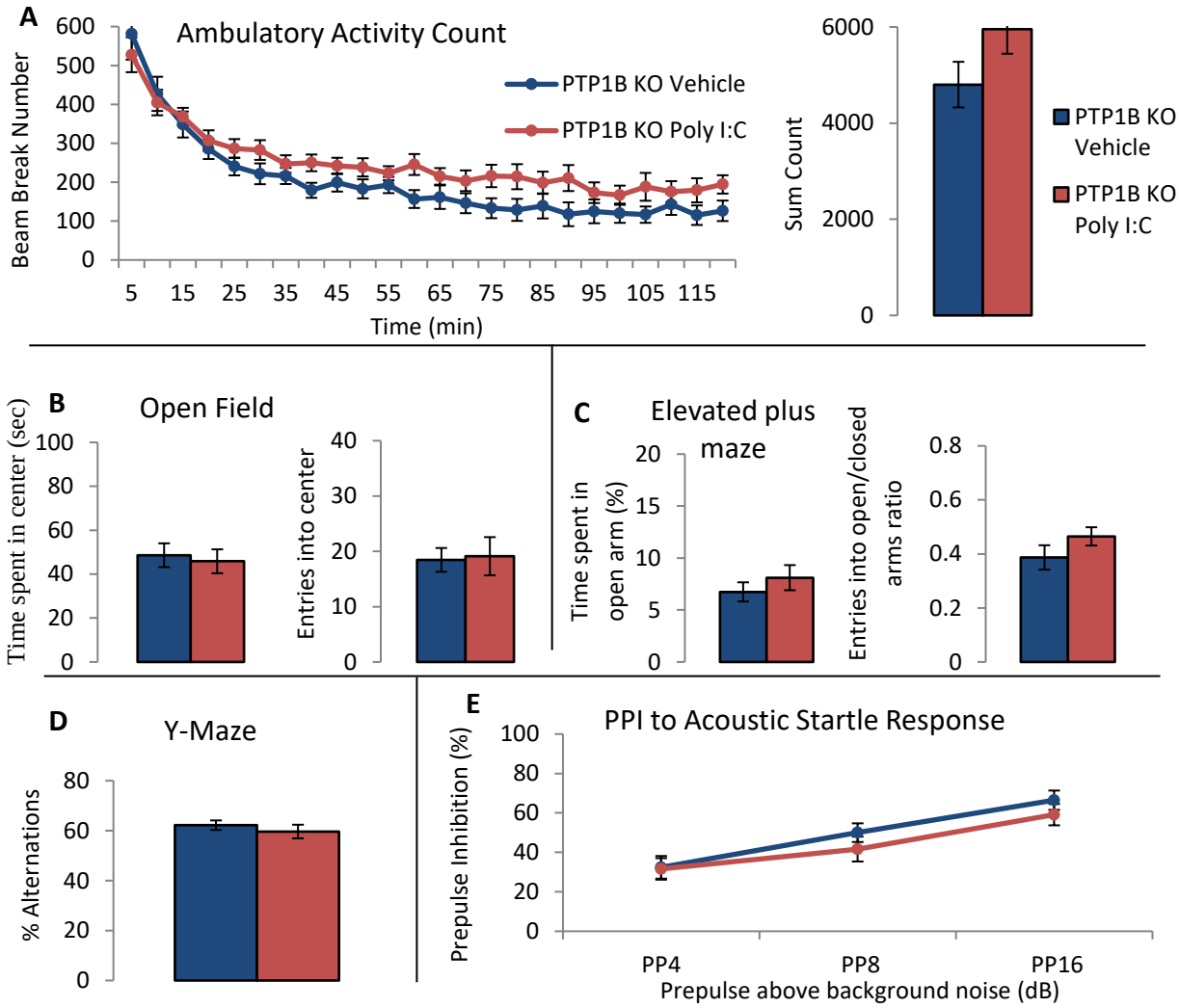


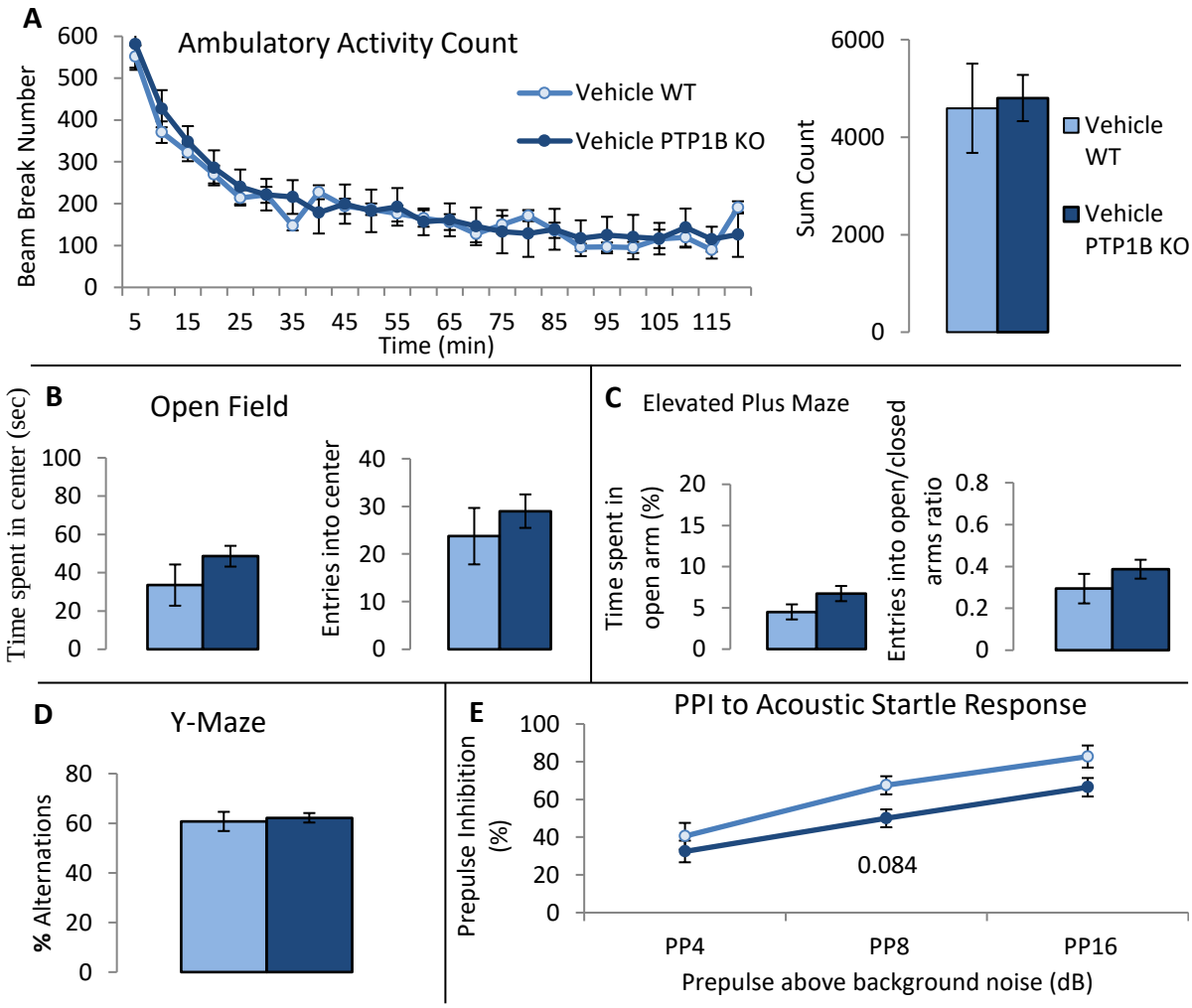
Figure 4. Neuronal knock out of PTP1B increases horizontal movement but has no effect on anxiety, working memory and sensory gating of male mice.

A. Time course and sum of ambulatory (sequential) beam break number for vehicle treated males. B. Time and entries into open field center. C. Percent time in elevated plus maze open arms and entries into open/closed arms. D. Male percent alterations in Y-Maze. E. Prepulse inhibition for a prepulse 4, 8, and 16 dB above background noise. n=12, 17 vehicle treated male mice. Data are presented as mean ± SEM. *p<0.05; **p<0.005; ***p<0.0005; by student t-test.



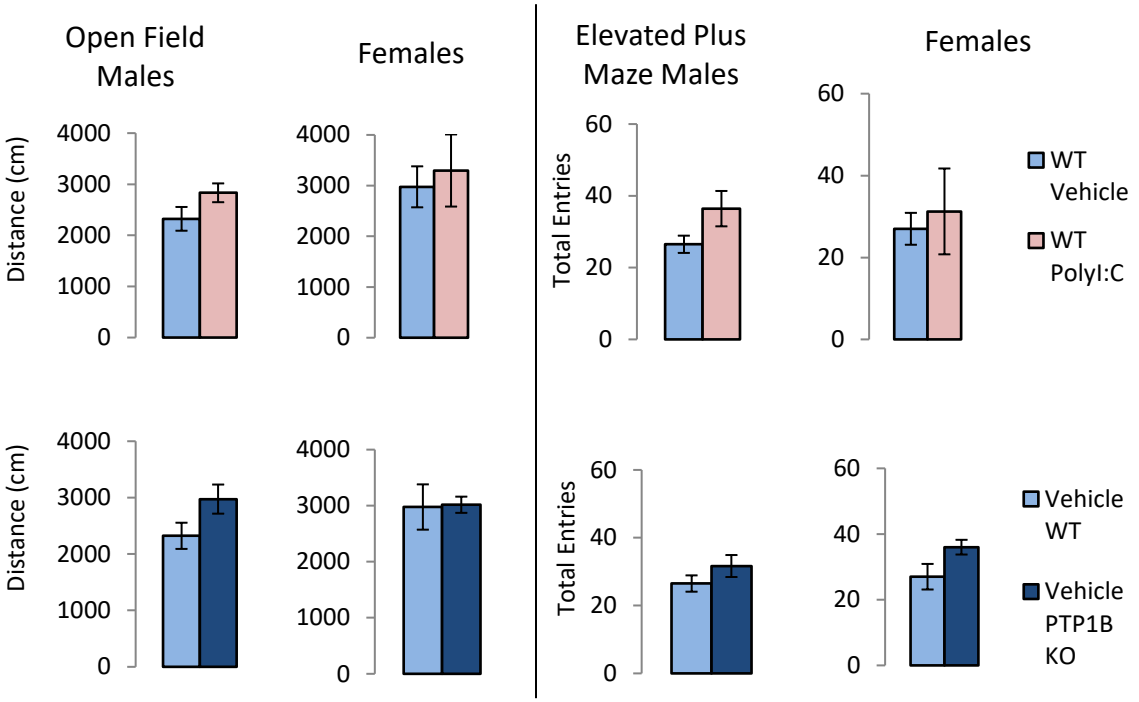
Supplementary Figure 1. PTP1B KO female mice do not demonstrate any changes due to prenatal treatment with poly I:C in horizontal movement, anxiety, working memory and sensory gating.

A. Time course and sum of ambulatory (sequential) beam break number for PTP1B KO males. B. Time and entries into open field center. C. Percent time in elevated plus maze open arms and entries into open/closed arms. D. Male percent alterations in Y-Maze. E. Prepulse inhibition for a prepulse 4, 8, and 16 dB above background noise. n=13, 14 PTP1B KO male mice. Data are presented as mean ± SEM. *p<0.05; by student t-test.

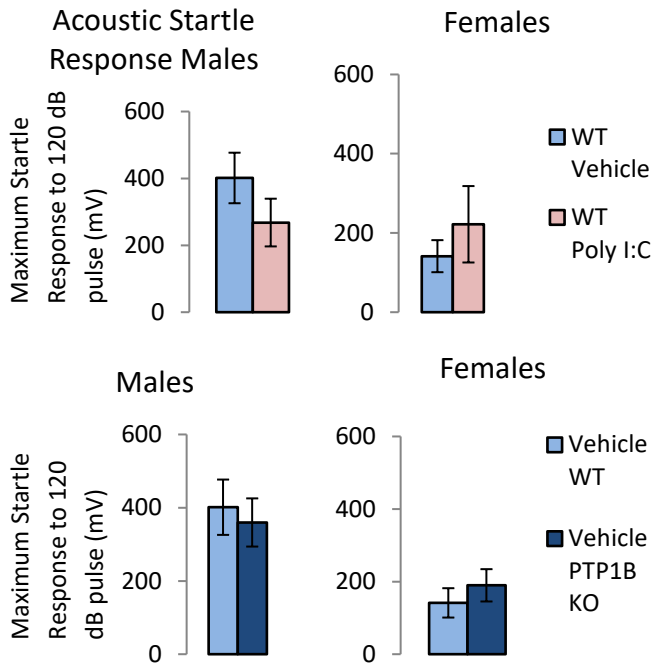


Supplementary Figure 2. Neuronal knock out of PTP1B has no effect on locomotor activity anxiety, working memory and sensory gating of female mice.

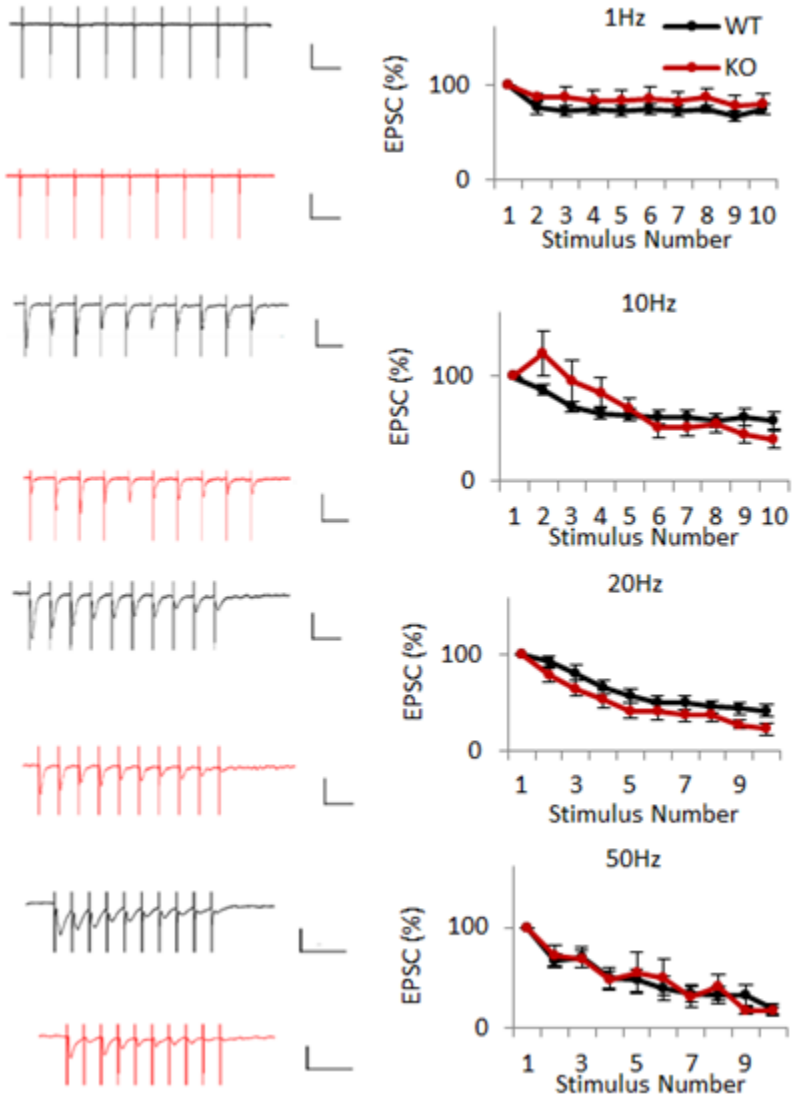
A. Time course and sum of ambulatory (sequential) beam break number for vehicle treated males. B. Time and entries into open field center. C. Percent time in elevated plus maze open arms and entries into open/closed arms. D. Male percent alterations in Y-Maze. E. Prepulse inhibition for a prepulse 4, 8, and 16 dB above background noise. n=4, 13 vehicle treated male mice. Data are presented as mean ± SEM. *p<0.05; **p<0.005; ***p<0.0005; by student t-test.



Supplementary Figure 3. Prenatal treatment with Poly I:C or conditional neuronal knockout of PTP1B does not affect distance traveled on open field test or total entries onto open and closed arms of elevated plus maze for both male and female mice.
Distance traveled in open field test and total entries on open and closed arms of elevated plus maze test. n=4, 18 mice. Data are presented as mean ± SEM. *p<0.05; by student t-test.

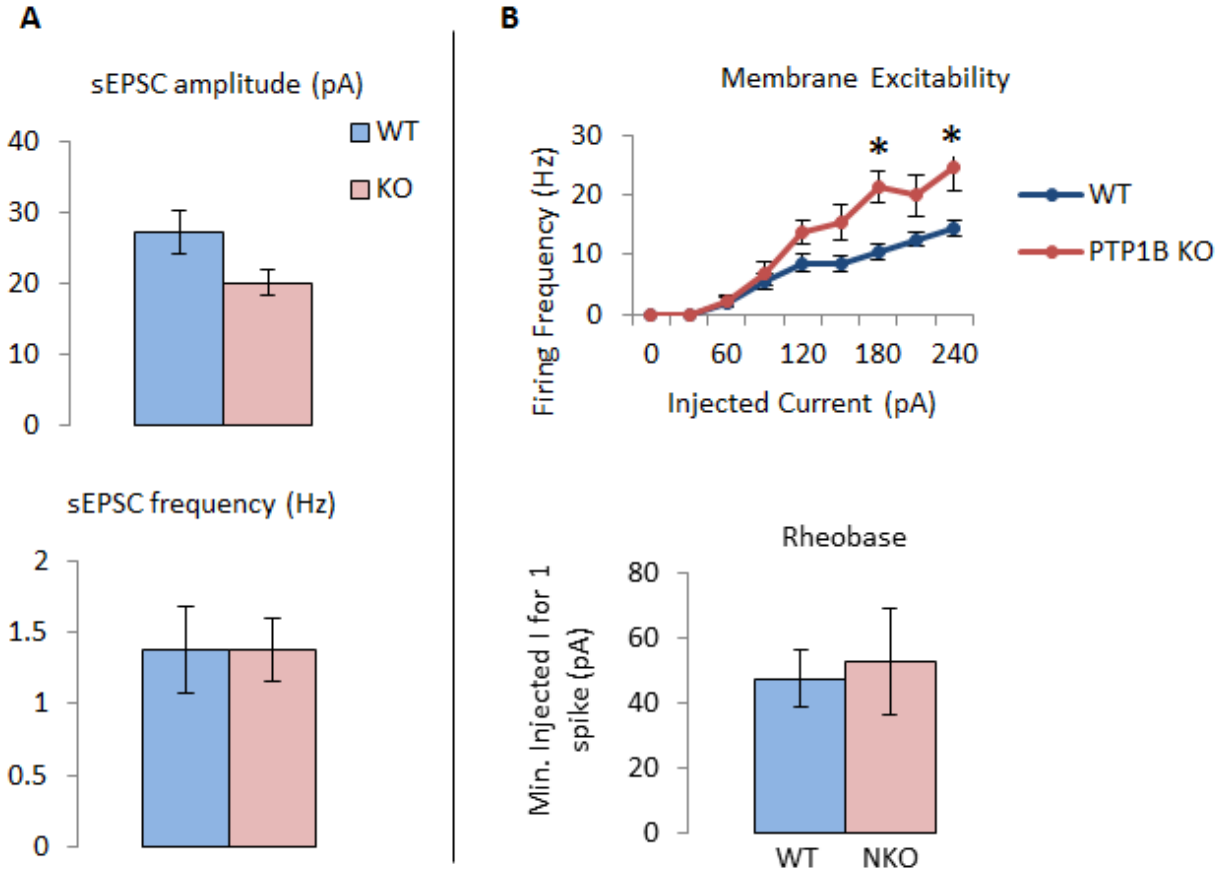


Supplementary Figure 4. Acoustic startle response is unchanged due to prenatal treatment with Poly I:C or because of conditional neuronal knock out of PTP1B.
Peak response measured due to startle response due to acoustic 120 dB pulse. n=4, 18 mice. Data are presented as mean ± SEM. *p<0.05; by student t-test.

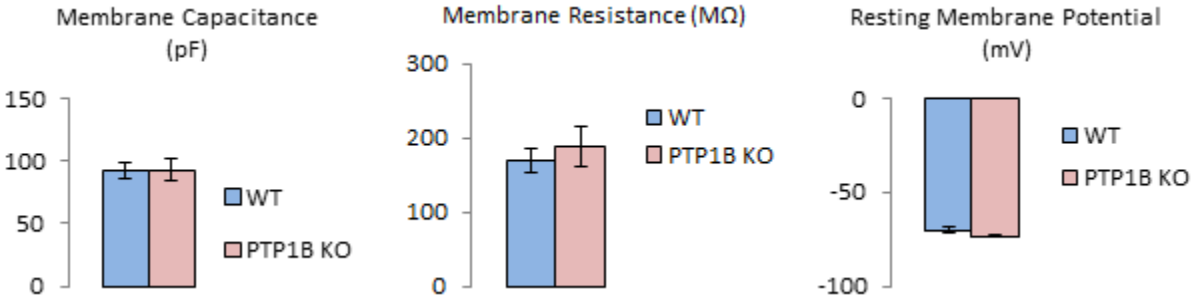


Supplementary Figure 5. No significant difference in basolateral amygdala pyramidal neuron presynaptic properties between WT and PTP1B KO male mice under basal conditions.

Example traces and paired-pulse ratio for pulses at 50, 100, 250, and 500 ms (n=20, 22 cells) For 1 Hz, vertical bar =100pA, horizontal bar=1000ms; 10Hz, vertical bar=120pA, horizontal bar=100ms; 20Hz, vertical bar=70pA, horizontal bar=75ms; 50Hz, vertical bar=70pA, horizontal bar=120ms. Data are presented as mean ± SEM. *p<0.05; by student t-test.



Supplementary Figure 6. Altered postsynaptic properties of basolateral amygdala pyramidal neurons in male mice.
A. sEPSC amplitude and frequency (n=12, 12 cells). B. Firing frequency for specific injected currents (n=14, 20 cells) and minimum injected current to induce a single spike (rheobase, n=5, 8 cells). Data are presented as mean ± SEM. *p<0.05; by student t-test.



Supplementary Figure 7. Membrane properties of cells measured in membrane excitability experiment.
Membrane capacitance, resistance, and resting potential. (n=14, 20 cells) and minimum injected current to induce a single spike (rheobase, n=5, 8 cells). Data are presented as mean ± SEM. *p<0.05; by student t-test.

Supplementary Methodology

Electrophysiology tests were performed 4-6 weeks after birth.

Whole-cell patch clamp recordings were performed on pyramidal neurons (selected from morphology and electrophysiological properties) of the basolateral amygdala from 300μm thick organotypic brain slices in artificial cerebral spinal fluid (119 mM NaCl, 2.5mM KCl, 1.3mM MgSO, 1mM NaHPO, 2.5mM CaCl, 26.2 NaHCO, 25mM glucose). Potassium gluconate internal solution (115mM K Gluconate, 20mM KCl, 2mM MgCl2, 10mM HEPES, 4mM Na+ ATP, 10mM Na Phosphocreatine, 0.3mM GTP) was used for current-clamp experiments (excitability and rheo base). For voltage-clamp experiments (paired-pulse ratio, train pulse, sEPSC), we used a cesium chloride internal solution (115mM Caesium Methane sulfonate, 0.4mM EGTA, 5mM TEA-Cl, 2.8mM NaCl, 20mM HEPES, 3mM Mg ATP, 0.5mM GTP, 10mM Na phosphocreatine). Membrane potential was maintained at -70 mV for voltage-clamp experiments and stimulations were sent laterally to the patched neuron with appropriate intensity to cause excitatory postsynaptic currents (EPSC) with amplitudes ranging from 50 to 200 pA. For paired-pulse ratio experiment, 100μs pulses were sent at 50, 100, 250 and 500 ms intervals. The rates of train pulse experiments were 1, 10, 20, and 50 Hz.

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