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The effect of the molecular weight of poly(I:C) on the development and behavior of offspring in the maternal immune activation model

Vliv molekulové hmotnosti poly(I:C) na vývoj a chování potomstva v modelu mateřské imunitní aktivace

Diploma thesis

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Prohlášení:

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Abstract

Maternal inflammation during pregnancy is associated with an increased risk of the development of psychiatric disorders in offspring, especially schizophrenia and autism. Prenatal treatment with poly(I:C), a viral mimetic, is a widely used model of maternal immune activation leading to developmental and behavioral alterations in offspring. However, results of studies are inconsistent possibly due to the differences in the molecular weight of used poly(I:C). In this thesis, the effect of different molecular weights of poly(I:C) was assessed. Pregnant Wistar rat females were treated by either high molecular weight (HMW) or low molecular weight (LMW) poly(I:C) on gestational day 14 to assess developmental and behavioral deficits relevant to schizophrenia and autism in offspring on postnatal day 12 and at three months. Prenatal exposition to the HMW poly(I:C) led to significantly reduced social behavior in the Homing test and a trend towards reduced USV vocalization in pups. The LMW pups showed significantly impaired negative geotaxis. In adulthood, the HMW and LMW offspring both exhibited significant social deficits and reduced anxiety. Anxiety was reduced mainly in the LMW group. This thesis revealed differences in behavioral outcomes between prenatal exposition to HMW and LMW poly(I:C). These outcomes were predominantly relevant to autism spectrum disorder. Present results will be important during the planning of experiments and for assessing past scientific literature.

Keywords: maternal immune activation, poly(I:C), molecular weight, psychiatric diseases, schizophrenia, autism

Abstrakt

Zánět v těle matky během těhotenství je spojen se zvýšeným rizikem rozvoje psychiatrických poruch u potomků, zejména schizofrenie a autismu. Prenatální aplikace poly(I:C), virového mimetika, je hojně používaným modelem mateřské imunitní aktivace vedoucí k vývojovým a behaviorálním změnám potomků. Výsledky studií jsou však nekonzistentní, zřejmě kvůli rozdílům v molekulové hmotnosti použitého poly(I:C). V této práci byl hodnocen vliv různé molekulové hmotnosti poly(I:C). Březím samicím potkana kmene Wistar bylo aplikováno 14. den gestace vysokomolekulární (HMW) nebo nízkomolekulární (LMW) poly(I:C) k zhodnocení vývojových a behaviorálních deficitů související se schizofrenií a autismem u potomků během postnatálního dne 12 a po dovršení tří měsíců. Prenatální expozice HMW poly(I:C) vedla k významně sníženému sociálnímu chování v Homing testu a trendu snížené USV vokalizace u mláďat. Mláďata LMW vykazovala významně narušenou negativní geotaxi. V dospělosti vykazovali potomci HMW a LMW významné sociální deficity a sníženou úzkost. Úzkost byla snížena především ve skupině LMW. Tato práce odhalila rozdílné účinky prenatální expozice HMW a LMW poly(I:C) na chování. Tyto výsledky se týkaly převážně poruchy autistického spektra. Současné výsledky budou důležité při plánování experimentů a hodnocení minulé vědecké literatury

Klíčová slova: mateřská imunitní aktivace, poly(I:C), molekulová hmotnost, psychiatrická onemocnění, schizofrenie, autismus

Outline

List of Abbreviations

5-HT	serotonin
ANCOVA	analysis of covariance
ANOVA	analysis of variance
AP-1	activator protein 1
ASD	autism spectrum disorder
CNS	central nervous system
dsRNA	double-stranded ribonucleic acid
Fig	figure
GABA	γ-aminobutyric acid
GD	gestational day
GH	growth hormone
HMW	high molecular weight
IFN	interferon
IGFI	insulin-like growth factor
IKK	IκB kinase
IL	interleukin
IRF	interferon regulatory factor
JAK	janus kinase
JNK	c-Jun N-terminal kinase
LMW	low molecular weight
LPS	lipopolysaccharide
m.o.	month old
MAPK	mitogen-activated protein kinase
MKK	mitogen-activated protein kinase kinase
MIA	maternal immune activation
MK-801	dizocilpine
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	N-methyl-d-aspartate

PD	postnatal day
poly(I:C)	polyinosinic:polycytidylic acid
PPI	prepulse inhibition
RNA	ribonucleic acid
SAL	saline
SEM	standard errors of the mean
SPF	specific pathogen-free
STAT	signal transducer and activator of transcription
TLR	toll-like receptor
TNF	tumor necrosis factor
TRAF	tumor necrosis factor receptor-associated factor
TRIF	toll-IL-1 receptor domain-containing adapter-inducing interferon
TUNL	Trial-unique Nonmatching-to-Location task
UID	Unified Information Device
USV	ultrasonic vocalization

1. Introduction

The understanding of the etiology of psychiatric diseases is still limited. Two of the prevalent disorders that elude understanding are schizophrenia and autism. However, several lines of evidence indicate that both these disorders have a developmental origin (reviewed in: Patterson, 2002, 2009). It was proposed that prenatal exposure to maternal immune activation is associated with altered brain physiology in offspring, due to the strong link between maternal viral infections during pregnancy and the elevated incidence of schizophrenia and autism spectrum disorders later in life (Takei et al., 1996; Patterson, 2002; Shi & Patterson, 2005). Early human studies observed this elevated incidence in the offspring of mothers pregnant during major influenza epidemics (Sham et al., 1992; Adams et al., 1993; Takei et al., 1996). It was later proposed that the influenza virus affects the fetal brain indirectly, likely as a result of maternal immune system activation and inflammation, as a study by Shi & Patterson (2005) showed that the viral RNA was not detected in mice fetuses. As research on prenatal infections is often unavailable in humans, researchers have developed multiple maternal immune activation (MIA) models in animals to investigate the causality of the relationship (Cui et al., 2009; Deleidi et al., 2010; Rose et al., 2017). One of the most widely used MIA models is induced by an injection of a viral mimetic, a synthetic double-strand RNA polyinosinic:polycytidylic acid [poly(I:C)], to pregnant rodents. The poly(I:C) MIA model is reported to model schizophrenia-like and autism-like behaviors (reviewed in: Meyer & Feldon, 2012; Reisinger et al., 2015; Haddad et al., 2020a). Unfortunately, results of studies are not consistent, most probably due to differences in protocols; it is therefore challenging to interpret the results. This reduces the robustness and replicability of the model. One of the possible sources of variability can be a difference in the molecular weight of poly(I:C) used between studies. The different molecular weights of poly(I:C) were shown to have different effects on immune response in pregnant females (see later). Poly(I:C) products can differ in terms of the relative amount of dsRNA fragments (i.e., the length of the poly(I:C) chain) (Mueller et al., 2019). As of the time of writing this thesis, the molecular weight of poly(I:C) is not always precisely specified by the manufacturer, and random mixtures of poly (I:C) are being sold even in preparations sold from the same vendor. In addition, most of the studies do not verify or report the composition of poly(I:C) used. Refinement and unification of the poly(I:C) model protocols could contribute to the research of the influence of maternal immune activation on the development of psychiatric disorders and lead to improvement in the clinical treatment and prevention strategies of psychiatric disorders (Mueller et al., 2019; Kowash et al., 2019).

2 Literature Review

2.1 Maternal Inflammation and Human Neuropsychiatric Disorders

Maternal inflammation during pregnancy has been associated with the development of psychiatric disorders in the offspring; most often with autism spectrum disorders (ASD) and schizophrenia (Takei et al., 1996; Patterson, 2002, 2009; Atladóttir et al., 2010; Lee et al., 2015). Recent studies also point to a possible link between maternal inflammation and attention deficit hyperactivity disorder (Gustafsson et al., 2020), major depression (Gilman et al., 2016) as well as bipolar disorder (Parboosing et al., 2013). Inflammation in mothers with a history of psychiatric disorders is believed to further increase the risk of developing psychiatric disorders in the offspring (Blomström et al., 2016).

The mother's immune system can be challenged by factors that produce an either acute, chronic, or autoimmune immunological response. All of the above have been previously discussed and associated with an increased psychiatric diagnosis in offspring, including schizophrenia and ASD (Benros et al., 2011; reviewed in Bennett & Molofsky, 2019; Ellul et al., 2022). Factors triggering the immune response include infectious agents typically causing a rapid acute inflammatory response in the organism characterized by the site-specific infiltration and increase of immune cells (e.g., neutrophils, macrophages, monocytes) and an elevation of pro-inflammatory markers and cytokines (reviewed in: Kubes & Ward, 2000; Adams Waldorf & McAdams, 2013). Another factor, obesity, is associated with altered metabolism potentially causing chronic low-grade inflammation in the body. The inflammatory response in obesity, however, differs from the acute inflammatory response. Obesity can reprogram immune cells to support a pro-inflammatory environment, including increasing pro-inflammatory cytokines in adipose tissues, and is subsequently chronically sustained by, for example, adipocytes (Pantham et al., 2015; Denizli et al., 2022). Obesity is closely linked to intestinal dysbiosis, which can also contribute to chronic low-grade systemic inflammation. Dysbiosis causes impaired protection against pathogenic agents and bacterial penetration in response to increased intestinal permeability, which results in an expression of pro-inflammatory cytokines and other inflammatory markers (Lam et al., 2012; Yamashiro et al., 2017). The increased levels of pro-inflammatory cytokines and altered immune cell function have been associated with multiple types of stress (Hantsoo et al., 2019).

The activation of the mother’s immune system, by any of the above-mentioned means, triggers through the placenta a systemic inflammatory response in the fetus (as illustrated in

Figure 1), which may negatively affect the development of fetal organs, particularly the brain, with potentially long-lasting effects that may persist postnatally (Benros et al., 2011; reviewed by Rivera et al., 2015; Bennett & Molofsky, 2019; Ellul et al., 2022). An increase in pro-inflammatory cytokines is widely considered a culprit that is associated with a negative effect on the fetus (see next chapters) (Dahlgren et al., 2006; Smith et al., 2007; Hsiao & Patterson, 2011; Adams Waldorf & McAdams, 2013). This work is concerned with the link between maternal inflammation and the risk of ASD and schizophrenia in offspring.

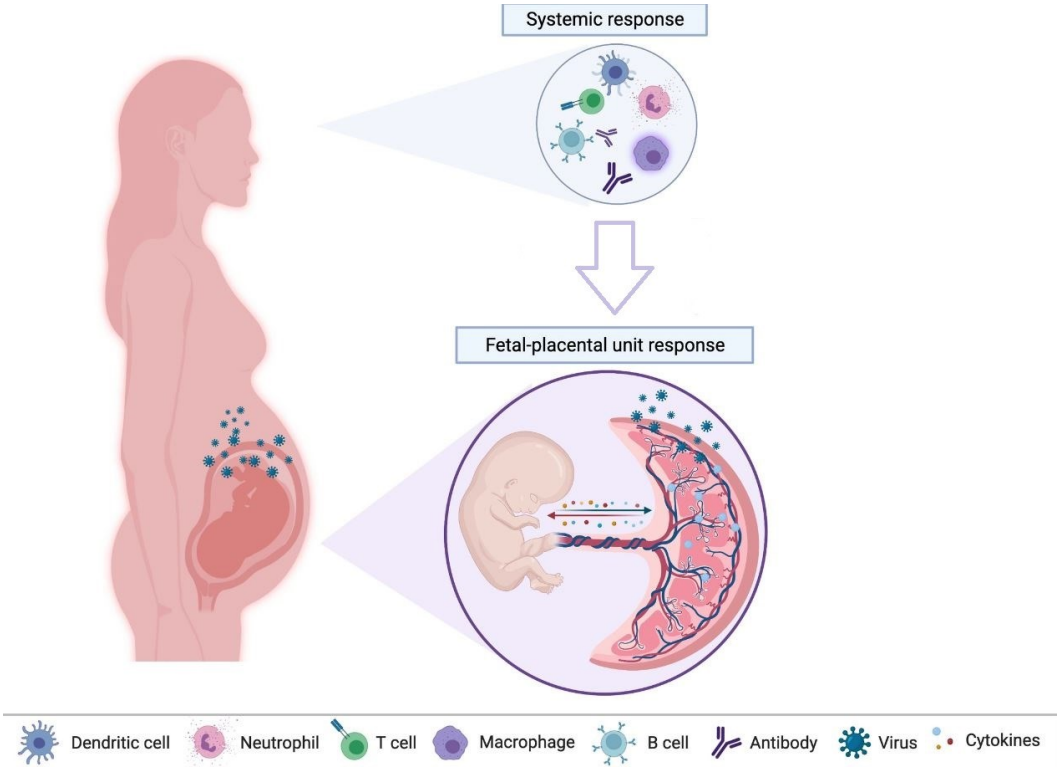

Fig.1: Maternal immune reaction in pregnancy after exposition to a virus. An infection, for example by a virus, during pregnancy causes an immune response in the mother's body. The maternal immune response can subsequently alter the function of the placenta and increase the expression of pro-inflammatory cytokines in the fetal-placental unit, which can lead to altered fetal development (adapted and modified from Ding et al., 2022).

2.1.1 Schizophrenia and Autism Spectrum Disorders

Acute maternal inflammation is mostly associated with schizophrenia and ASD (Takei et al., 1996; Patterson, 2002, 2009; Atladóttir et al., 2010; Lee et al., 2015).

Schizophrenia is a multifaceted neurodevelopmental psychiatric disorder characterized by several symptom clusters and subclusters. The symptoms of schizophrenia are most commonly classified into positive symptoms (such as hallucinations and delusions), negative symptoms (such as lack of motivation and social isolation), and cognitive symptoms (such as working memory deficits) (reviewed in McCutcheon et al., 2020). The peak age of schizophrenia onset of both men and women is early in life (16 - 20 years of age) and later in life (41 - 45 years of age). Another middle peak age of onset is observed mainly in women (26 - 30 years of age) (Selvendra et al., 2022). The two-hit hypothesis suggests the role of genetic and non-genetic factors in schizophrenia onset. The first hit in the two-hit hypothesis could be a genetic abnormality and the second hit could be the influence of environmental factors during life (social stress, infections, etc.) (Bayer et al., 1999). It was later proposed that inflammatory processes may substitute for the first hit, pointing to the influence of maternal inflammation during pregnancy on the schizophrenia diagnosis later in life (Feigenson et al., 2014). The two-hit hypothesis was supported by animal models (illustrated in Figure 2), including the poly(I:C) MIA model (Chamera et al., 2021; Guerrin et al., 2021). The role of the immune system in schizophrenia is emphasized by significantly different cytokine levels in the cerebrospinal fluid in schizophrenia patients compared to healthy controls (Miller et al., 2011; Gallego et al., 2018; Wang & Miller, 2018). Overall, schizophrenia development may be strongly connected to immune system alterations.

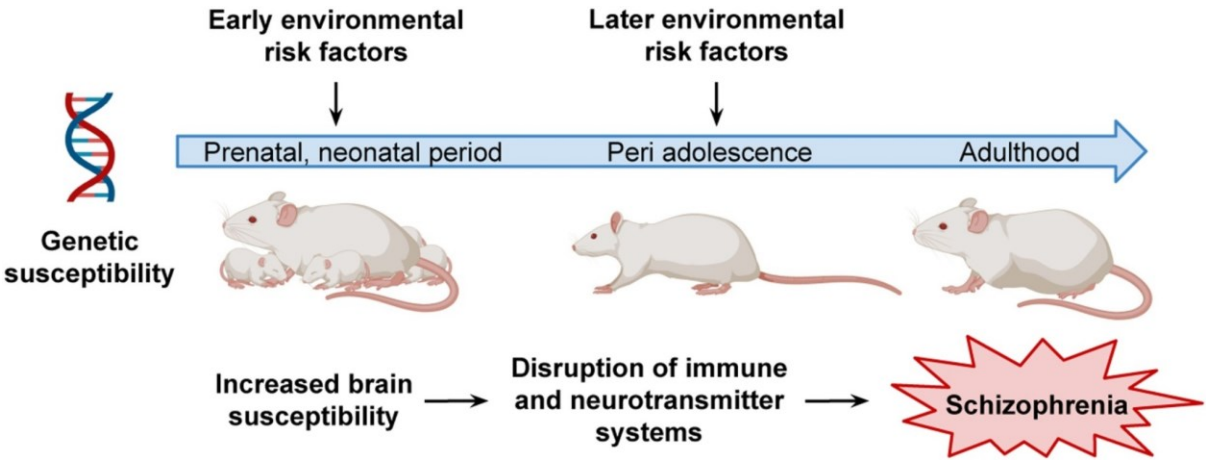

Fig.2: Schematic representation of the two-hit hypothesis of schizophrenia. The environmental or genetic risk factors early in life, the first hit, can alter the immune and neurotransmitter systems (such as dopaminergic, GABAergic, etc.), respectively. This could lead to increased brain susceptibility to other environmental risk factors appearing later in life (peri adolescence). A second hit, such as social stress or drug use in humans, could provide additional disruption of the immune and neurotransmitter systems and cause cognitive and behavioral changes (schizophrenia-like) in adulthood (adapted from Guerrin et al., 2021).

ASD is another neuropsychiatric disorder widely discussed in connection to maternal inflammation. ASD is characterized by severe impairment in social interaction, communication, and restricted and/or stereotyped behavior (reviewed in Volkmar & Pauls, 2003). The peak age of onset is in early childhood (around 5 years of age) (Solmi et al., 2022). Multiple studies emphasized the role of the immune system in ASD incidence. For example, disbalances of immune functions (inflammatory markers, antibody levels, cytokines, etc.) were observed in autistic patients and associated with the severity of ASD symptoms (Al-Ayadhi & Mostafa, 2012; Manzardo et al., 2012; Goines & Ashwood, 2013; Masi et al., 2017). Furthermore, asthma and allergy in women during pregnancy have been associated with increased incidence of ASD in offspring highlighting the role of maternal immunity (Croen et al., 2005). The altered expression of inflammatory molecules in ASD patients was supported by animal studies as well. Neuroimmune alterations in animal models were strongly linked to autism phenotypes (Wei et al., 2012; Choi et al., 2016; Bagnall-Moreau et al., 2020). In conclusion, similarly to schizophrenia, ASD development is likely significantly linked to the maternal immune system.

2.1.2 Limitations in Human Studies

Due to ethical and practical considerations, research involving human subjects is largely limited (Kapp, 2006). Therefore, the research in human subjects largely consists of epidemiological and empirical studies, which, indeed, first revealed an association between maternal inflammation and psychiatric disorders in offspring (Sham et al., 1992; Takei et al., 1996). However, these studies did not necessarily prove causality and did not reveal mechanisms behind maternal infection and psychiatric diseases later in life (Meyer & Feldon, 2012). Thus, well-defined and reliable animal models are necessary to better understand the molecular mechanisms underlying psychiatric and neurodevelopmental changes in offspring exposed to maternal inflammation (Lipska & Weinberger, 2000; Meyer & Feldon, 2012).

2.1.3. Utility of Animal Models

Animal models are commonly used in neuroscience research to investigate specific aspects of neuropsychiatric disorders, such as schizophrenia and ASD (Kapp, 2006; Crawley, 2012; Nikiforuk, 2018). These animal models generally employ homogenous populations of subjects in well-defined and controlled experimental conditions (Meyer & Feldon, 2012; Crawley, 2012; Nikiforuk, 2018). Models of neuropsychiatric disorders are developed using genetic manipulations (Hikida et al., 2007), surgeries (McDannald et al., 2011), or pharmacological treatments (Su et al., 2022). Researchers attempting to model human psychiatric disorders in animals have always been dealing with skepticism, as it is nearly unachievable to replicate the complex symptomatology of human psychiatric disorders (such as hallucinations and delusions in the case of schizophrenia) in animals. However, focusing on selected behavioral phenotypes can successfully approximate the symptoms of these disorders (Lipska & Weinberger, 2000; Meyer & Feldon, 2012). To characterize animal models in a more systematic way, animal models are evaluated based on the fulfillment set of criteria called validities. There are three validities: face, predictive, and construct. Face validity addresses the similarity between the animal's behavioral phenotype and the human symptom profile. Predictive validity evaluates and compares the ability of models to respond to human treatments as well as to predict concrete markers of disorders. Construct validity evaluates the similarity of the neurobiological basis between the model and disordered humans. These criteria help to evaluate to what extent

the model truly models the given human disease (Belzung & Lemoine, 2011; Reisinger et al., 2015; Czéh & Simon, 2021).

2.2. Animal Models of Maternal Immune Activation

To identify mechanisms and connections between maternal inflammation and the development of neurodegenerative disorders in offspring, multiple animal models of maternal immune activation (MIA) were developed (Cui et al., 2009; Deleidi et al., 2010; Rose et al., 2017). The first animal MIA models were conducted in 1999 and in the early 2000s by exposing pregnant mice to the human influenza virus (Fatemi et al., 1999; Shi et al., 2003; Fatemi et al., 2004). Next, researchers have developed models focusing on the role of imbalances and interactions between maternal and fetal cytokines as a possible key mechanism of pathological brain abnormalities in the offspring (Meyer et al., 2009). MIA induced by an injection of a viral mimetic, polyinosinic:polycytidylic acid [poly(I:C)], to pregnant rodents (usually rats or mice), is one of the most commonly used models for this purpose (Deleidi et al., 2010; Meyer & Feldon, 2012; Mueller et al., 2021). Other common MIA models are using the administration of bacterial endotoxin lipopolysaccharide (LPS) (Romero et al., 2007), bacterial superantigens (such as staphylococcal enterotoxin A) (Glass et al., 2019), and an injection of selected individual cytokines, for example IL-6 (Smith et al., 2007). All these MIA models examine the maternal effect on the fetus by challenging the immune system of the dams using different methods.

2.2.1 Maternal Immune Activation Using Poly(I:C)

This diploma thesis will focus on the MIA model using poly(I:C) administration, as it is recognized as a valid model in several aspects. The poly(I:C) MIA model brings the advantage of a high degree of face validity as well as solid predictive and construct validity, especially regarding schizophrenia-like and autism-like pathology (Meyer & Feldon, 2012; Reisinger et al., 2015; Haddad et al., 2020a). The abnormal behavioral phenotypes associated with schizophrenia and ASD, such as social deficits (Malkova et al., 2012; Chamera et al., 2021; Su et al., 2022), impaired working memory (Murray et al., 2017; Gogos et al., 2020), or impaired sensorimotor gating (Wolff & Bilkey, 2008; Wolff & Bilkey, 2010), have been reported in offspring after the prenatal poly(I:C) treatment supporting the face validity of the poly(I:C)

model. Several neuropsychiatric deficits, such as social deficits (Okamoto et al., 2018) or working memory deficits (Meyer et al., 2010), in offspring evoked by the poly(I:C)-induced MIA were reversed by drug treatment, therefore a fair degree of predictive validity for a model of schizophrenia and ASD was recognized (Meyer & Feldon, 2012; Reisinger et al., 2015). The solid construct validity of the poly(I:C) model lies in the replicability of a common risk factor in pregnancy (Haddad et al., 2020a). Next to that, a strong feature of the poly(I:C) model is the precise timing of MIA. This can help to determine specific developmental periods sensitive to prenatal maternal infections (Meyer & Feldon, 2012; Gray et al., 2019; Haddad et al., 2020a, Haddad et al., 2020b). In summary, poly(I:C) is a valid model of schizophrenia and ASD.

There are variations in the administration of poly(I:C) to pregnant dams. Differences between protocols occur in the route of administration, GD of administration, and dose of poly(I:C). Poly(I:C) is most often administered by a single intraperitoneal (Kowash et al., 2022), intravenous (Meehan et al., 2017), or subcutaneous (Ferreira et al., 2020) injection. Some studies administered poly(I:C) for several days in a row (Arsenault et al., 2014; Volk et al., 2015). Doses of poly(I:C) vary between studies, ranging from 0.25 mg/kg to 20 mg/kg (reviewed in Hameete et al., 2020). The 5-20 mg/kg dosage is most commonly used in rat and mouse studies resulting in behavioral abnormalities in offspring (Buschert et al., 2016; Gray et al., 2019; Haida et al., 2019; Kowash et al., 2022; Su et al., 2022).

Poly(I:C) is usually administered to pregnant rat dams on gestational day (GD) 14 or 15 (Gray et al., 2019; Chamera et al., 2021; Kowash et al., 2022; Su et al., 2022), with the aim to disrupt extensively developing dopaminergic system, which occurs during this period (Santana et al., 1992; Meyer et al., 2008a; Gray et al., 2019). However, in some studies, poly(I:C) was administered to rats in earlier or later gestational phases, such as during GD10 or GD19 (Meehan et al., 2017; Rahman et al., 2020). The choice of gestational day of the MIA by poly(I:C) seems to be critical to induce certain abnormal behavioral phenotypes in the offspring. Working memory impairments were more frequently observed in rat offspring exposed to late MIA (e.g., GD19) than to early MIA (e.g., GD10) (Meehan et al., 2017). The increased startle response is more likely observed in adult rats prenatally treated on GD9.5 compared to rats treated on GD14.5 (Haddad et al., 2020b). Open Field exploration was reduced in GD 9 but not on GD 17 (Meyer et al., 2006). Apart from the differences, which may arise by choosing a particular gestation day for poly(I:C) treatment, the interpretation of the results is also thwarted by differences in naming methodology: there is no clear consensus on

how to mark the day of conception (e.g., GD0 x GD0.5 x GD1) as well as the day of birth (e.g., PD0 x PD0,5 x PD1), which may create some confusion in the timing of the MIA.

2.2.2 Verification of Poly(I:C)-Induced MIA

The verification of poly(I:C)-induced MIA confirms successfully initiated immune response in the body of pregnant females. Several methods can be used for the verification, such as observation of sickness behavior (Cunningham et al., 2007, Foster et al., 2021) core body temperature (Kentner et al., 2019; Foster et al., 2021), and plasmatic cytokine concentration measurements (Mueller et al., 2019).

One of the above-mentioned methods verifying the immune challenge in mothers are the observational methods of sickness behavior (e.g., decrease in reward-seeking and motivation, piloerection), including food intake and weight loss. The sickness behavior can be evaluated by using a sickness score system, a five-point scale (from 0-4) rating the presence of four sickness behaviors of the animal during a time period (Kolmogorova et al., 2017). These methods are non-invasive and reduce handling stress to a minimum, however, they are also the least accurate and precise (Cunningham et al., 2007; Hopwood et al., 2009; Foster et al., 2021).

Another method used to verify the immune response after poly(I:C) administration is the measurement of core body temperature. Several studies have observed a temperature elevation (i.e., fever response) in dams treated by poly(I:C) compared to controls a few hours (at up to 6.5 h) after the injection (Fortier et al., 2004; Hopwood et al., 2009; Foster et al., 2021). When the temperature elevation following the poly(I:C) administration occurs, it is considered to induce MIA in the body of a pregnant dam (Cunningham et al., 2007; Foster et al., 2021). The temperature changes can be monitored by several tools. These tools include rectal thermometers (Dangarembizi et al., 2017), infrared cameras (Qu et al., 2020), implantable telemetry (Fortier et al., 2004; Missig et al., 2018), and temperature-sensitive microchips (Foster et al., 2021), each having its advantages and limitations. For example, despite being a non-invasive temperature measurement method, a rectal thermometer might not be a suitable tool for monitoring maternal body temperature after poly(I:C) injection, as it reportedly causes stress-induced hyperthermia, providing false positives and inaccurate recording (Dangarembizi et al., 2017). The infrared camera is another non-invasive device for temperature measurement; however, the detection depth of the recording is not as deep as desirable (Qu et al., 2020). Implantable telemetry is a rather costly and invasive approach, as

it requires surgery and subsequent recovery, which could influence the MIA (Kentner et al., 2019). On the other hand, the temperature programmable microchips are considered to be a cheaper and less invasive alternative: microchips are smaller, can be implanted subcutaneously using an injector, and animals require only a short recovery period due to the small size of the injury (Redfern et al., 2017; Foster et al., 2021).

Alternatively, it is possible to examine the presence of MIA by measuring plasmatic concentrations of pro-inflammatory cytokines (IL-6, TNFα, etc.), which appears to be the most precise method (Mueller et al., 2019). Nevertheless, the collection of blood samples exposes animals to the risk of inflammation caused by blood extraction-related injury, as well as to handling stress. Temperature measurement as well as cytokine concentration measurement may lead to unwanted immune activation in both treated and control animals, which may confound experimental outcomes (Kentner et al., 2019). Altogether, it is reasonable to quantify the degree of maternal response to verify the effect of poly(I:C), which can be assessed by several approaches that have different benefits and risks (invasiveness, costs, etc.).

2.2.3 The Pregnancy Outcomes After Poly(I:C) Administration

One of the drawbacks of poly(I:C), and other MIA models, is that they can significantly influence the outcome of the animal's pregnancy. Multiple research groups presented results showing maternal body weight loss after injecting animals with poly(I:C) suggestive of a partial abortion of the litter (Kowash et al., 2019; Brown et al., 2022). The research in mice of Mueller et al. (2018) connected the prenatal timing of the MIA (i.e., the specific GD) to the increased risk of spontaneous litter abortion rates. A poly(I:C) treated mouse in earlier GDs of the pregnancy was documented to be more prone to litter abortion than in later GDs. It is suggested that the pregnancy outcomes are also dose-dependent e.g., lower poly(I:C) doses are associated with a low immune response, while higher doses are linked to the increased immune response leading to a greater incidence of litter abortions (Mueller et al., 2018; Estes et al., 2020), or can even cause death to the animal (Homan et al., 1972). However, Mian et al. (2013) proposed that it is really the length of the poly(I:C) chain which matters to evoke differential cytokine responses, not the prenatal timing or doses (see Chapter 2.4.2).

2.3 Molecular Effects of Prenatal Poly(I:C) Exposure

Poly(I:C) causes changes at the molecular, cellular, and systemic levels of the fetal organism, and these changes, individually or in combination, can lead to neurodevelopmental and behavioral changes in offspring (Dahlgren et al., 2006; Smith et al., 2007; Hsiao & Patterson, 2011; Mueller et al., 2021, Su et al., 2022). Poly(I:C) is a synthetic double-strand RNA, which selectively binds to Toll-like receptor 3 (TLR-3) (Alexopoulou et al., 2001). Toll-like receptors are a family of receptors mediating innate immunity and are essential for microbial recognition distinguishing between specific motifs of microbial components (Akira, 2001; Takeda & Akira, 2001). The signaling pathway of TLRs activates innate immunity, which leads to the forming of adaptive immunity, which is antigen-specific (Takeda & Akira, 2001). TLRs are localized in many tissues in the body (Herath et al., 2006; Huang et al., 2018; Mallaret et al. 2022) as well as in the CNS of humans, rodents, and other vertebrates (Bsibsi et al., 2002; Iqbal et al., 2005; Bao et al., 2022). TLRs in the CNS are broadly expressed by microglia, astrocytes, and oligodendrocytes (Bsibsi et al., 2002) but also by neurons (Préhaud et al., 2005). They are situated mainly in plasma membranes but can be also found in the membranes of endosomes, as in the case of TLR-3 (Bsibsi et al., 2002; Matsumoto & Seya, 2008).

The binding of poly(I:C) to TLR-3 induces an expression of pro-inflammatory cytokines e.g., tumor necrosis factor-alpha (TNF-α), interleukins IL-1β, IL-6, and type I interferons (IFNs) in microglia (Olson & Miller, 2004). In astrocytes, activation of TLR-3 leads to an expression of anti-inflammatory cytokines contributing to the neuroprotective function of these cells (Bsibsi et al., 2006; Kim et al., 2008), however, an expression of pro-inflammatory TNF-α, IFN-β (Park et al., 2006) and IL-6 (Kim et al., 2008) was also reported in astrocytes after the TLR-3 activation. In neurons and oligodendrocytes, the role of TLR-3 activation in the promotion of a pro-inflammatory environment is not clear.

The pro-inflammatory cytokine expression, especially in microglia, is induced through Toll-IL-1 receptor domain-containing adapter-inducing interferon-β (TRIF)-dependent signalization pathway. This pathway is activating the formation of nuclear factor kappa B (NF-κB), activator protein 1 (AP-1) transcription factor complex, and interferon regulatory factor 3 (IRF3) (Alexopoulou et al., 2001; Hsiao & Patterson, 2011; Careaga et al., 2018, Yan et al., 2019). A simplified overview of the TLR-3 signaling pathway can be found in Figure 3. For a detailed review of the molecular mechanisms of poly(I:C) action see Matsumoto & Seya (2008) or Bao et al. (2022), as this topic is beyond the scope of this thesis.

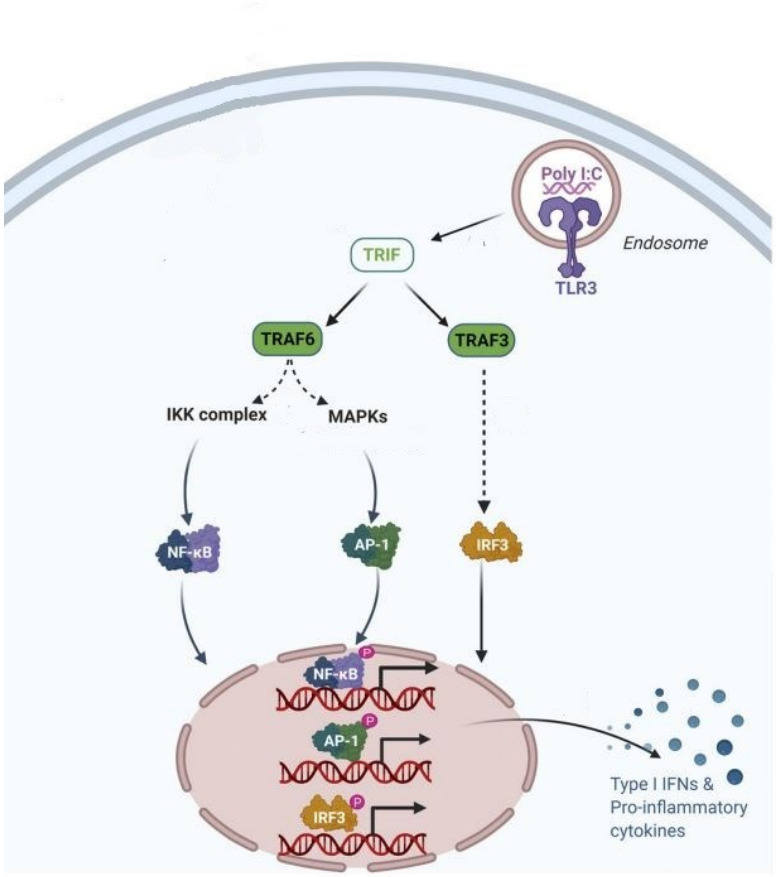

Fig.3.: Schematic representation of immune activation by poly(I:C) through TLR3 pathway. Poly(I:C) activates TLR3, which leads to the activation of the TRIF-signaling pathway and, subsequently, to the activation of tumor necrosis factor receptor-associated factor-6 (TRAF6) and tumor necrosis factor receptor-associated factor-3 (TRAF3). TRAF3 activation induces the formation and phosphorylation of IRF3. IκB kinase (IKK) complex and mitogen-activated protein kinases (MAPKs), activated by TRAF6, induce the NF-κB dimer and AP-1 transcription factor complex formations, which are, together with IRF3, afterward translocated into the nucleus. As a result, the expression of pro-inflammatory genes is elevated (adapted and modified from Bao et al., 2022).

2.3.1 IL-6 and Other Pro-Inflammatory Cytokines

The elevation of cytokine production by poly(I:C) caught the attention of researchers focusing on MIA for several reasons (Yamamoto et al., 2003; He et al., 2011; Brown et al., 2022). In the first place, it is possible that elevated maternal cytokines after poly(I:C) administration translocate into the placenta and alter its function (Dahlgren et al., 2006; Smith et al., 2007; Hsiao & Patterson, 2011; Mueller et al., 2021). Recent studies on rodents suggest that the

maternal immune response disrupts amino acid transport from the placenta to the fetus, causing essential amino acid deficiency (McColl & Piquette-Miller, 2019; Kowash et al., 2022). In addition, it was also suggested that poly(I:C)-induced MIA leads to up-regulated gene expression of pro-inflammatory cytokines directly in the placenta and the yolk sac, and, subsequently, in the fetal brain; possibly affecting fetal neurodevelopment in the process. Fetal brain damage can then lead to abnormal behavior in the animal (Smith et al., 2007; Hsiao & Patterson, 2011; Choi et al., 2016; Kowash et al., 2022), as already mentioned in the previous chapter (2.2.1). The link between altered cytokine production after poly(I:C)-induced MIA and the abnormal behavior of animals, which is simulating the symptoms of human psychiatric disorders is plausible, as disbalances in cytokine levels were observed in autistic (Al-Ayadhi & Mostafa, 2012; Manzardo et al., 2012; Goines & Ashwood, 2013) as well as schizophrenia patients (Miller et al., 2011; Gallego et al., 2018; Wang & Miller, 2018) in several studies (mentioned in chapter 2.1.1).

IL-6 is one of the most widely investigated pro-inflammatory cytokines in relation to the poly(I:C) MIA model. Using methods of genetic mutants and blocking antibodies revealed that especially IL-6 is strongly connected to ASD-like and schizophrenia-like behavior, e.g., social or sensorimotor gating deficits (Smith et al., 2007). Next to that, it was proposed that elevated maternal IL-6 by poly(I:C) administration decreases levels of placental growth hormone (GH) and insulin-like growth factor 1 (IGFI) via the JAK/STAT3 pathway (Hsiao & Patterson, 2011) as indicated in Figure 4. Significantly lower concentrations of IGF-I were previously reported in the cerebrospinal fluid of children with ASD (Vanhala et al., 2001).

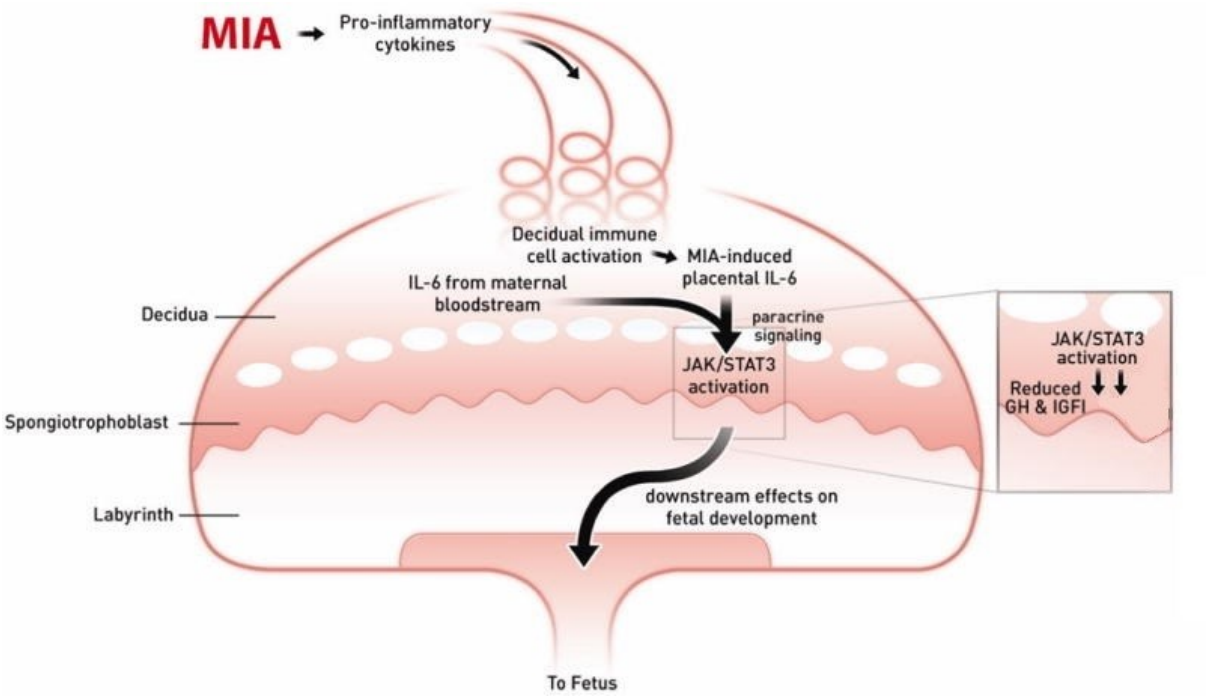

Fig.4: Schematic representation of IL-6 effects in the placenta after MIA. After MIA by poly(I:C), pro-inflammatory cytokines, such as IL-6, are secreted in the maternal bloodstream and circulate through the placenta. Increased IL-6 and other pro-inflammatory factors in the arteries pass through the decidua, spongiotrophoblast, and the labyrinth, subsequently circulating back to the maternal compartment. The maternal cytokines and signaling factors activate the decidual immune cells, promoting the additional release of cytokines, including IL-6. The IL-6 then signals to the cells in the spongiotrophoblast in a paracrine manner, which leads to JAK/STAT3 activation and gene expression changes. Increased levels of acute phase proteins cause down-regulation of GH expression in the placenta, therefore, reducing GH signaling, which subsequently causes the decrease of IGFI levels. These processes supposedly alter the development of the fetus (adapted and modified from Hsiao & Patterson, 2011).

Among other maternal cytokines involved in the development of deficits in the fetal brain are, for example, IL-17a and IFNs type I (Choi et al., 2016; Ben-Yehuda et al., 2020). A study by Choi et al. (2016), using again methods of genetic mutants and blocking antibodies, showed that MIA resulted in abnormal cortical development in mouse fetuses, which was mediated by IL-17a. They suggested that the IL-17a pathway is closely related to ASD-like phenotypes in poly(I:C) offspring manifesting in behaviors such as abnormal ultrasonic vocalization, social interaction deficits, or repetitive behavior. At the same time, maternal IL-17a was shown to be dependent on IL-6 levels suggesting some degree of overlap in their

influence on behavior and indicating possible interactions between cytokines (Pratt et al., 2013; Choi et al., 2016). In addition, a recent study also implies that maternal IFNs type I, which are also elevated by poly(I:C) exposure, could crucially affect the brain of the mouse fetus, presumably by affecting microglial development (see chapter 2.3.2) (Ben-Yehuda et al., 2020). In summary, the disbalance in cytokine levels and cytokine response as well as all the changes in the placenta could potentially influence neurodevelopment and induce significant behavioral and transcriptional changes in the offspring.

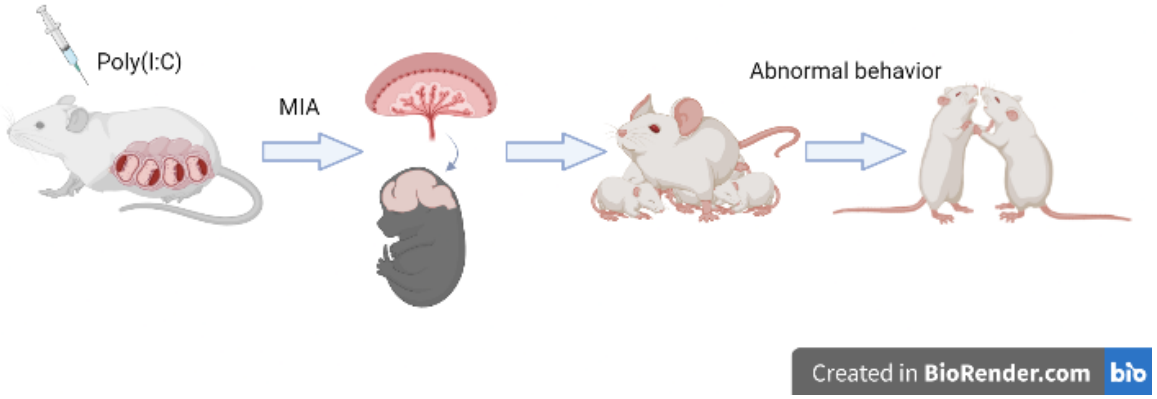

Fig.5: Schematic representation of poly(I:C)-induced MIA model. After the injection of poly(I:C) to pregnant dams, the MIA causes crucial changes altering the placental function and subsequently affecting the neurodevelopment of the fetal brain. This could lead to abnormal behavior in offspring in early-postnatal days as well as in adulthood (Smith et al., 2007; Hsiao & Patterson, 2011; Mueller et al., 2021).

2.3.2 Changes at the Cellular Level

Poly(I:C)-induced MIA also causes changes at the cellular level of the organism. These changes involve microglia, Purkinje cells, and hippocampal place cells.

Poly(I:C) reportedly alters and modulate the density, morphology, and function of microglia in MIA offspring (Juckel et al., 2011; Zhu et al., 2014; Murray et al., 2019; Ben-Yehuda et al., 2020). The activation of microglia accompanying neuroinflammation was observed in humans in ASD as well as schizophrenia patients and was characterized by e.g., increased cell density, morphological changes, and shifts in their reactivity (for reviews see Laskaris et al., 2016; Liao et al., 2020; Rodrigues-Neves et al., 2022). Microglial alterations are therefore possibly linking MIA to the development of neuropsychiatric disorders. For example, Ben-Yehuda et al. (2020) proposed that poly(I:C)-induced MIA could represent the

"first hit" in the two-hit hypothesis of schizophrenia (mentioned in chapter 2.1.1) by disrupting microglial development, leading to greater sensitivity to postnatal stress in mouse offspring. The occurrence of higher levels of stress in the offspring may later be the "second hit". Moreover, a study by Chamera et al. (2021) on rats reported that MIA evoked by poly(I:C) alters the expression of the neuron-microglia proteins (CX3CL1-CX3CR1) in the frontal cortex as well as in the hippocampus of adult offspring rats. They speculate that these alterations could insinuate a shift in the reactivity of microglial cells and modify the immune response after the "second hit" in the two-hit hypothesis, and therefore be partially responsible for behavioral abnormalities in adult rat offspring.

In addition to microglial cellular changes, poly(I:C) reportedly influence the number of Purkinje cells. Aavani et al. (2015) reported increased numbers of Purkinje cells in the cerebellum of poly(I:C) mouse offspring suggesting impaired cellular death of these cells. This study also emphasizes the role of microglia in this process. However, Haida et al. (2019) reported contradictory results, showing a reduced number of Purkinje cells in the cerebellum of poly(I:C) rat offspring. It is hypothesized that changes in the number of Purkinje cells could result in sensorimotor deficits in poly(I:C) offspring. On top of that, changes in the cellular number and morphology of Purkinje cells were previously found in schizophrenia patients (Tran et al., 1998; Maloku et al., 2010; Mavroudis et al., 2017). Moreover, schizophrenia patients display motor coordination deficits that most likely stem from cerebellar dysfunction (Rathod et al., 2020).

Next, Wolff & Bilkey (2015) reported that hippocampal place cells are another cellular type influenced by poly(I:C)-induced MIA. Specifically, their results showed altered spatial firing (reduced place field size) of place cells in adult poly(I:C) offspring, without the disturbance of the basic place cell firing properties. Changes in the communication of place cells may result in behavioral abnormalities linked to spatial memory disruption in poly(I:C) offspring. Spatial memory deficits were also previously reported in schizophrenia (Fajnerová et al., 2014) and autistic (Lind et al., 2013) patients. Overall, poly(I:C)-induced MIA seems to be crucially involved in cellular survival, morphology as well as function in multiple cell types.

2.3.3 Changes in Neurotransmitter Systems

Changes in glutamatergic (Amodeo et al., 2019), GABAergic (Canetta et al., 2016), dopaminergic (Su et al., 2022), and serotonergic (Holloway et al., 2013) transmission were all observed following MIA induced by poly(I:C).

A study by Roenker et al. (2011) in mice reported significantly increased basal levels of extracellular glutamate in the prefrontal cortex in poly(I:C) offspring. Furthermore, Amodeo et al. (2019) showed that poly(I:C)-induced MIA decreased expression of genes involved in glutamatergic neurotransmission, especially of metabotropic glutamate receptor 7, in mouse offspring. The disruption in the glutamatergic neurotransmission is closely associated with schizophrenia and ASD in patients (reviewed in Eltokhi et al., 2020) and was also reported in animal models of these disorders (Hayashi-Takagi et al., 2010; Wei et al., 2015; Lander et al., 2019; Moutin et al., 2021). Higher levels of glutamate were found in patients with schizophrenia and associated with the severity of this disorder (Bustillo et al., 2021, Merritt et al., 2016) as well as in patients with autism (Shinohe et al., 2006). In addition, Amodeo et al. (2019) reported down-regulated expression of mTOR (mammalian target of rapamycin) signaling genes in poly(I:C) offspring. Their findings correspond to changes in mTOR signalization in schizophrenia patients (Chadha et al., 2021).

A study by Canetta et al. (2016) found that MIA by poly(I:C) leads to decreased GABAergic transmission in parvalbumin-expressing interneurons in the medial prefrontal cortex, which was linked to increased anxiety-like behavior of adult mouse offspring. Moreover, Okamoto et al. (2018) reported physiological hyperactivity of neurons in the anterior cingulate cortex due to the hypofunction of GABAergic synapses in poly(I:C) mouse offspring (for illustration see Figure 6). This dysfunction could be responsible for some of the reported deficits in social behavior as this behavior normalized after the enhancement of $GABA_A$ receptors transmission by clonazepam in the anterior cingulate cortex. Corresponding to that, lower levels of GABA have been reported in the brain of patients with ASD (Schür et al., 2016) as well as schizophrenia (Kumar et al., 2021).

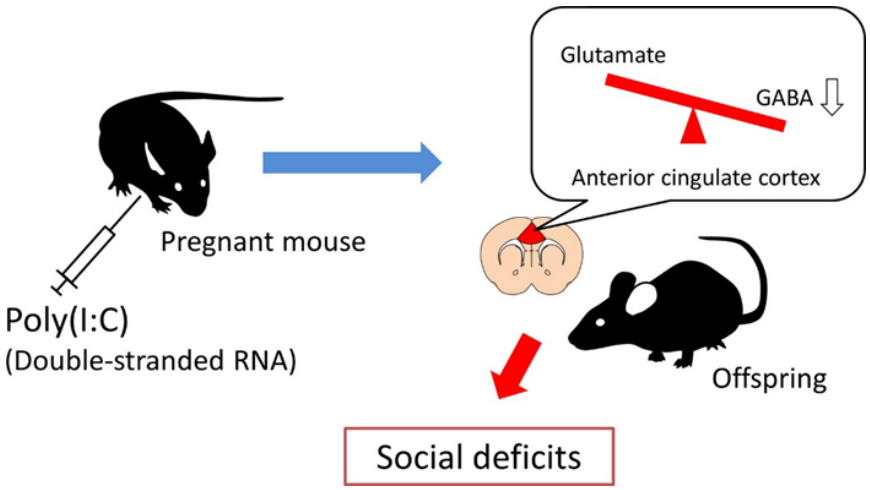

Fig.6: Schematic representation of the effect of MIA by poly(I:C) administration on the balance between GABA and glutamate in the offspring. After poly(I:C) administration to a pregnant mouse, the anterior cingulate cortex in offspring is characterized by abnormal activity of neurons induced by the hypofunction of GABAergic synapses causing an imbalance between glutamate and GABA. This imbalance is suggested as the cause of social deficits of poly(I:C) offspring (adapted from Okamoto et al., 2018).

As already mentioned, MIA induced by poly(I:C) is associated with changes in the dopaminergic system, and these changes mainly involve site-specific changes in D2 receptor gene expression. A recent study reported that MIA by poly(I:C) resulted in increased expression of dopamine D2 receptor in the nucleus accumbens (Su et al., 2022), and the cortex (Buschert et al., 2016) in offspring. On the other hand, decreased expression of the D2 receptor was observed in the hippocampus in poly(I:C) rat offspring (Su et al., 2022). The altered dopaminergic system is closely linked to both schizophrenia and ASD and the severity of their symptoms (Uchida et al., 2009; Perez & Lodge, 2012; Chhabra et al., 2023). The D2 receptor is a common target of schizophrenia antipsychotic treatment (Potkin et al., 2014; Simpson et al. 2022).

Next, poly(I:C)-induced MIA led to serotoninergic (5-HT) system impairments. Goeden et al. (2016) reported that MIA upregulates tryptophan metabolism and its conversion to 5-HT in the mouse placenta and results in increased levels of 5-HT in the brain of the poly(I:C) fetuses, which could consequently alter 5-HT-dependent processes. Moreover, a study by Holloway et al. (2013) in mice showed the up-regulation of 5-HT2A receptors in the frontal cortex of adult poly(I:C) offspring. As the serotonergic system is linked to several

psychiatric disorders, including schizophrenia, major depression, and ASD (for reviews see Shimizu et al., 2013; Gabriele et al., 2014; Ślifirski et al., 2021), the connection of MIA models is plausible. Specifically, elevated levels of 5-HT were observed in the blood of ASD patients (Gabriele et al., 2014), which partially corresponds to the study of Goeden et al. (2016). In summary, poly(I:C)-induced MIA leads to the disruption of neurotransmitter systems and synaptic function, which are closely linked with schizophrenia and ASD in humans.

2.4 The Outcomes of MIA by Poly(I:C) on the Behavior of Offspring Relevant to Neurodevelopment, Schizophrenia, and Autism.

Many studies have linked MIA by poly(I:C) to a range of specific behavioral abnormalities in offspring, which are often contradictory or manifest differently across studies (Gray et al., 2019; Horváth et al., 2019; Gogos et al., 2020; Talukdar et al., 2020; Su et al., 2022). Due to the similarity between symptoms of schizophrenia and autism in humans, and poly(I:C)-induced behavioral changes in animals, the abnormal behavior in animals is commonly referred to as schizophrenia-like or autism-like. Schizophrenia and autism-like behaviors in rodents are related to clusters of deficits in behavior and cognition (Crawley, 2012; Nikiforuk, 2018).

Impaired sensorimotor gating (Braff & Geyer, 1990; Weiss & Feldon, 2001), together with impaired latent inhibition (Baruch et al., 1988; Weiner & Arad, 2009) are one of the most stable phenotypes of schizophrenia and therefore one of the most common tests performed in animal models of schizophrenia (Weiss & Feldon, 2001; Weiner & Arad, 2009). The sensitivity and behavioral response to NMDA receptor antagonists (Kishi & Iwata, 2013; Nakazawa & Sapkota, 2020) and dopamine receptor agonists (Klawan & Margolin, 1975; Gray et al., 2019; Kusljic et al., 2022) are also commonly investigated in animal models in relation to schizophrenia. Cognitive symptoms of schizophrenia include deficits e.g., in working and spatial memory, attention, verbal and visual learning, as well as problem-solving (reviewed in Tripathi et al., 2018). All these cognitive domains are also explored in animal models of schizophrenia (Nikiforuk, 2018).

Impaired cognition is also relevant to autism-like behavior, as working and spatial memory impairments have been described not only in patients with schizophrenia (Dreher et al., 2001; Fajnerová et al., 2014; Lett et al., 2014) but also in patients with ASD (Lind et al., 2013). Other autism-like behaviors include repetitive behaviors, deficits in social interaction, and elevated anxiety (reviewed in Crawley, 2012; McCutcheon et al., 2020). All the above-

mentioned behaviors relevant to schizophrenia and autism were observed following MIA by poly(I:C).

The poly(I:C)-induced MIA is associated with the induction of impaired sensorimotor gating exhibited in the Prepulse Inhibition (PPI) test of acoustic startle responses (Wolff & Bilkey, 2008; Wolff & Bilkey, 2010). Moreover, the ability to disregard stimuli that are inconsequential in a given situation is supposedly disrupted in poly(I:C) offspring observed in the Latent Inhibition test (Zuckerman & Weiner, 2003; Meehan et al., 2017). Interestingly, Meehan et al. (2017) reported sensorimotor gating deficits only in male offspring.

The working memory disruption by poly(I:C) had been presented and assessed in e.g., the odor span test, which showed reduced odor span capacity of poly(I:C) adult offspring (Murray et al., 2017). Using Trial-Unique Non-matching to Location (TUNL) task, Gogos et al. (2020) reported fewer correct responses dependent on the increase in difficulty, indicating a deficit in spatial working memory, however only in male rats. A study on mice by Nakamura et al. (2021) revealed contradictory results.

Hyperactivity after amphetamine administration, an indirect dopamine-receptor agonist, is commonly used to assess psychosis-like behavior in animal models (Klawan & Margolin, 1975; Kusljic et al., 2022). Multiple studies indicate that poly(I:C)-induced MIA influenced the effects of amphetamine on the locomotion of offspring. Reduced locomotion and increased stereotyped repetitive behavior, specifically repetitive 360° body turns, after the amphetamine treatment of adult poly(I:C) mouse offspring were reported compared to saline controls (Buschert et al., 2016). In contrast with this study, other studies on rats and mice showed increased amphetamine-induced locomotor activity of poly(I:C) offspring (Meyer et al. 2008b; Vorhees et al., 2015; Gray et al., 2019).

In addition, higher locomotion response to NMDA receptor antagonists, such as MK-801 and ketamine, was observed in several MIA by poly(I:C) studies (Zuckerman & Weiner, 2005; Zavitsanou et al., 2014; da Silveira et al., 2017). NMDA antagonism exacerbates symptoms of schizophrenia, making the response to NMDA antagonists a good test to support the validity of schizophrenia models (Kishi & Iwata, 2013; Nikiforuk, 2018). Some studies provided evidence that poly(I:C) offspring show higher locomotor responses to the administration of MK-801 (Zuckerman & Weiner, 2005; Zavitsanou et al., 2014). Higher locomotion response was also observed after the administration of a subanesthetic dose of ketamine to offspring of poly(I:C) treated pregnant mice (da Silveira et al., 2017).

A well-documented behavioral change in the offspring of MIA-treated rats is the deficit in social behavior. Several studies conducted social tests, which showed decreased sociability, social communication, and social novelty behavior of rats and mice born to poly(I:C)-treated dams (Malkova et al., 2012; Talukdar et al., 2020; Su et al., 2022). Unlike the other studies mentioned above, Malkova et al. (2012) reported decreased social behavior only in male offspring. Contradictory results were shown by Gzieło et al. (2023) reporting an increase in social behavior in male poly(I:C) offspring. An increase in aggressive behavior of adult Wistar rat offspring in the Social Interaction test was observed as well (Chamera et al., 2020). Another study by the same group using the same methodological approach (GD, dose and administration route, etc.), showed decreased aggressivity of Sprague-Dawley rats, indicating potential differences in the effect of MIA between individual rat strains and species as well (Chamera et al., 2021).

Social communication, as another form of social behavior, can be tested by the Ultrasonic Vocalization (USV) test. In pups, USV is commonly measured after isolation from the mother, while in adults USV is measured during social interaction. Reduced USV calls (number and duration) were shown both in early postnatal life and adulthood of offspring after poly(I:C)-induced MIA in several studies (Hsiao et al., 2013; Malkova et al., 2012; Carlezon et al., 2019; Potasiewicz et al., 2020). Unlike the other mentioned studies, Carlezon et al. (2019) showed that the reduced number of USVs in poly(I:C) pups was sex-specific and was observed only in males. In contrast to these studies, Schwartzer et al. (2013), reported an increase in the number of USV calls of the isolated pups prenatally treated with poly(I:C). It is possible, that the different results may be due to different working protocols and methodologies (GD, dose, etc.). In addition to USV, the impact of MIA on early postnatal development is also evaluated by other tests, for example by negative geotaxis test. Impaired negative geotaxis reflexes of mouse offspring were observed after triggering MIA with poly(I:C) (Arsenault et al., 2014).

Another frequently observed behavioral phenotype in MIA models is increased anxiety, which is not necessarily directly related to schizophrenia or autism (Gray et al., 2019; Horváth et al., 2019; Talukdar et al., 2020; Su et al., 2022). Adult offspring of poly(I:C)-treated rats reportedly show increased anxiety, which manifests by, for example, spending more time near the walls and in the corners of an open field arena (Talukdar et al., 2020; Su et al., 2022) or by spending more time in the closed arms of an elevated plus maze apparatus compared controls (Li et al., 2021; Su et al., 2022).

2.4.1 Inconsistencies Across Studies

The impact of poly(I:C)-induced MIA on the development and behavior of the offspring significantly varies across studies. This applies even if animals of the same species, strain, and age were used, and MIA was induced by the same concentration of poly(I:C) administered on the same GD. Furthermore, a study by Mueller et al. (2021) showed that poly(I:C) offspring exhibiting distinct behaviors had varying immunological profiles and transcription in cortical and subcortical brain regions. Unfortunately, this makes the comparison between studies challenging and at the same time reduces the robustness and replicability of the results (reviewed in Haddad et al., 2020a; Kowash et al., 2019; Mueller et al., 2021). It is necessary to identify sources of inconsistencies and standardize the MIA models. Several hypotheses have been postulated trying to explain the cause of the observed inconsistencies: housing differences (Mueller et al., 2018), differences in baseline immunoreactivity (Estes et al., 2020), genetic factors (Openshaw et al., 2019), type of verification of MIA (Kentner et al., 2019), and molecular weight of poly(I:C) (Kowash et al., 2019).

Mueller et al. (2018) investigated the influence of the caging system (the open cage and individually ventilated cage systems). Their study indicates that the potency to induce certain behavioral phenotypes of the MIA offspring is affected by the interaction of the caging systems with the prenatal timing of MIA and the dosing of poly(I:C).

The next factor that could influence the MIA and its intensity in the animal's body is paradoxically the mere verification of the immune response by the researcher. As already mentioned in the previous chapter (2.2.1), there are several methods used to verify the MIA and the poly(I:C) potency (Kentner et al., 2019). The temperature measurement methods such as implantable telemetry (Fortier et al., 2004; Kentner et al., 2019; Foster et al., 2021) and the measurement of plasmatic concentrations of pro-inflammatory cytokines (Mueller et al., 2019) are rather invasive and expose animals to stress, which could exacerbate the ongoing immune response to poly(I:C) or trigger an immune response in the control group (Grandin, 1997; Kentner et al., 2019).

The research group of Estes et al. (2020) suggested that the different baseline immunoreactivity of individual pregnant dams could be responsible for the varying MIA and induce different intensities of MIA effects on the fetus and its postnatal behavior. In the study by Openshaw et al. (2019), the interaction of maternal genetic factors with the intensity of MIA

was studied in detail; their results indicate a possible role of c-Jun N-terminal kinase (JNK) signaling molecules, including mitogen-activated protein kinase kinase 7 (MKK7), as mediators of the MIA impact on the fetus.

Another critical factor that could potentially cause inconsistent results across studies is the molecular weight of poly(I:C) (Kowash et al., 2019).

2.4.2 Poly(I:C) Molecular Weight

In general, there are significant discrepancies and unclear specifications across studies regarding the type and origin of the poly(I:C) used for the MIA experiments, especially of its molecular weight. Unknown/unreported differences in the molecular weight of poly(I:C) could be the reason for disagreement between the results of the effects of MIA on offspring between studies (Kowash et al., 2019; Mueller et al., 2019). Poly(I:C) is generally divided into two types according to its molecular weight – high (1,5 - 8kb) and low (0,2 - 1kb) molecular weight poly(I:C). These two types of poly(I:C) differ from each other in the length of dsRNA (Kowash et al., 2019).

Several studies indicate that high molecular weight (HMW) poly(I:C) is significantly more efficient in TLR-3 activation and subsequent immune response promotion in comparison to low molecular weight (LMW) poly(I:C) (Mian et al., 2013; Zhou et al., 2013, Mueller et al., 2019). The results of Mueller et al. (2019) demonstrated greater maternal as well as placental mouse immune response by HMW poly(I:C) compared to LMW poly(I:C) obtained from InvivoGen. It is indicated that although both poly(I:C)s lead to cytokine elevation (Mian et al., 2013; Careaga et al., 2018), the HMW poly(I:C) induces greater elevation of cytokines such as TNFα, IFN-γ, IL-1β, IL-6, in the blood of pregnant dams after the administration provided the same GD as LMW poly(I:C) administration. Specifically, only dams treated with HMW poly(I:C) exhibited increased IL-6 levels measured 3h and 6h after the poly(I:C) administration in comparison with control rats (Careaga et al., 2018). However, the data of Mueller et al. (2019) showed that there was no difference in the brain of mouse fetuses between HMW poly(I:C) and LMW poly(I:C) regarding the elevation of cytokine levels. In summary, HMW and LMW poly(I:C) could potentially induce different effects through varying immune response induction.

The molecular weight of poly(I:C) has been rarely specified by major manufacturers and vendors, although, at the time of writing this thesis, it is possible to purchase directly HMW and LMW poly(I:C) (e.g., from InvivoGen). Nevertheless, recent investigations showed that random mixtures of HMW and LMW poly(I:C) are still being sold, with considerable batch-to-batch variability even when obtained from the same source (Kowash et al., 2019; Mueller et al., 2019). Kowash et al. (2019) reported significant differences in the molecular weight of poly(I:C) obtained from the most common poly(I:C) vendors, specifically Sigma and InvivoGen. The molecular weight between batches was more uniform in InvivoGen poly(I:C) products (151–166 kDa) compared to the highly variable Sigma poly(I:C) batches (100–325 kDa). Moreover, the Sigma poly(I:C) led to significantly greater variability of maternal plasma IL-6 concentration in treated rats compared to the InvivoGen poly(I:C). Overall, the varying composition of sold poly(I:C) is a significant issue in the methodology of the poly(I:C)-induced MIA model.

The molecular weight of poly(I:C) is also crucially influencing the pregnancy outcomes, as already mentioned in Chapter 2.2.3. HMW poly(I:C) treatment resulted in maternal weight-loss of pregnant rats and a significant reduction in their litter size compared to LMW poly(I:C) (Kowash et al., 2019; Brown et al., 2022). The differences in batches of poly(I:C) potassium salt provided by the same supplier (Sigma-Aldrich) have been also linked to varying rates of spontaneous litter abortions (Mueller et al., 2019). In addition, rats injected with HMW poly(I:C) exhibited extensive sickness behavior (reduced feeding and drinking), whereas no significant differences from controls were observed in LMW animals (Careaga et al., 2018). All things considered, compared to LMW poly(I:C), HMW poly(I:C) lead to a more efficient TLR-3 activation, and subsequently a greater expression of maternal pro-inflammatory cytokines, which possibly leads to a stronger MIA causing noticeably varying outcomes of pregnancy.

3 Aims of the Project

● To induce and verify the model of maternal immune activation in dams by both HMW and LMW poly(I:C) during gestational day 14.

● To compare developmental deficits in pups on postnatal days 4, 12, and 21, and behavioral deficits on postnatal days 12, between offspring of HMW-treated, LMW-treated, and control rat dams.

● To compare performance in behavioral tasks relevant to schizophrenia and autism in adult offspring (3m.o.) of HMW-treated, LMW-treated, and control rat dams.

4 Materials and Methods

4.1 Animals, Breeding, and Maternal Manipulations

The breeding and maternal manipulations were conducted at the Institute of Physiology of the Czech Academy of Science, Prague. Female and male Wistar rats were obtained from AnLab, s.r.o. (Vídeňská 1083, Prague 4) at the age of 2 months. Upon arrival, animals were housed per two or three in transparent polyethylene cages in a specific-pathogen-free (SPF) animal holding room with controlled temperature and humidity (22 ± 1 °C, 50 %), and constant light-dark cycle (lights on: 06:00 AM–06:00 PM). Pups and older offspring were kept in the same room. Animals had access to the rodent-pelleted chow and water *ad libitum* throughout the entire study. The only instance was the access to the chow which was briefly restricted for adult offspring during the rewarded alternation in the T-maze task (see below), as this task required animals to quickly move through the apparatus and search for food. In this case, the daily food intake was limited so that the animals retained 90-95% of their original weight; the restricted food regime lasted for 7 days. The experiments were conducted in two runs (the first run in September; the second run in December).

Upon arrival, the rats underwent 10 days of acclimatization to the animal room; during this period, they were left undisturbed. Afterward, the rats were handled by the experimenter for five minutes daily for seven days. On the second day of handling, the female rats were briefly anesthetized by isoflurane (CHEMICAL IBÉRICA PV, S.L., Salamanca, Spain) inhalation and subcutaneously implanted with a Temperature Programmable Microchip (UCT-2112; 2,1mm x 12mm; Unified Information Devices (UID); 500 Park Ave, Lake Villa) into the nape of the neck using a Microchip Pistol Grip Injector with Ejector (UID, UPGI-Q). The temperature-sensitive microchips were used for temperature measurement following the treatment (see below).

The timed mating began after the handling period. Three days before timed mating, males were housed individually; some of the soiled bedding from male cages was dispersed in female cages to promote mating behavior. The evening before mating (between four to six pm) the vaginal smears from females were obtained using vaginal lavage and their estrus cycle stage was determined. In detail, female rats were gently restricted in a piece of cloth and vaginal smears were obtained by vaginal lavage with saline (approximately 200 ul) using a blunt Pasteur pipette. Samples were not dyed in any way. A light microscope was used to determine

the cycle phase by the relative abundance of different cell types which are present in the vaginal smear (nucleated epithelial cells, anucleated cornified cells, leukocytes) and by the presence of cell clusters (Marcondes et al., 2002). Only females in the late proestrus and estrus were introduced to males; the male counterpart was chosen randomly for each female rat. A maximum of two females were introduced to each male's cage. The day of mating (i.e., the day females were introduced to males) was referred to as gestational day (GD) 0. A weight gain of approximately 20% from GD 0 to GD 14 was considered as a successful pregnancy. The breeding led to 17 pregnant dams in total.

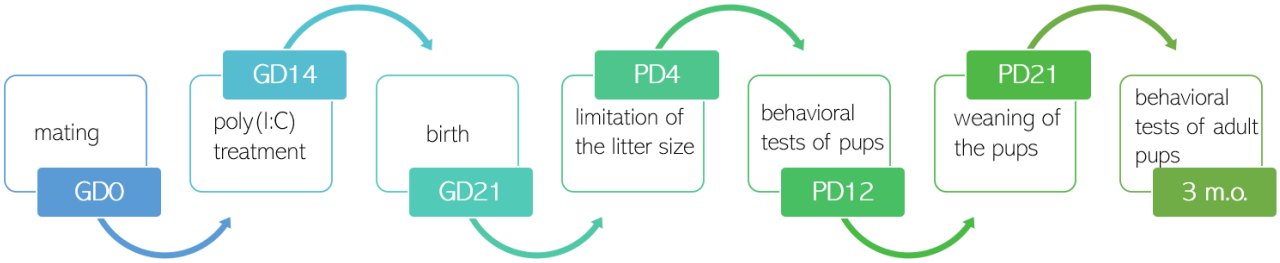

Fig.7: Timeline of the project

4.2 Prenatal Poly(I:C) Treatment and Temperature Measurement

Poly(I:C) treatment was administered on GD 14 to 17 pregnant dams in total. Before the treatment, dams were weighed, and their baseline temperature was determined using a microchip handheld reader URH-1HP (UID). Poly(I:C) solutions were prepared according to InvivoGen instructions by dissolving the lyophilized powder in 0.9% endotoxin-free NaCl and then heated to a temperature of 65°-70°C, then the solutions were aliquoted. Poly(I:C) solutions were prepared a few days before the treatment and kept in the freezer (in -20°C). The dams were then randomly assigned to a single subcutaneous injection into the groin (the inguinal region) of either low (LMW; cat.code: tlrl-picw; InvivoGen; 5, rue Jean Rodier, Toulouse, France) or high-molecular weight (HMW; cat.code: tlrl-pic-5; InvivoGen; Toulouse, France) poly(I:C), or to a vehicle solution (saline; endotoxin-free 0,9% NaCl, part of the package with poly(I:C); InvivoGen; Toulouse, France). Subcutaneous administration was chosen based on the experience in our department: we observed a higher abortion rate after the intraperitoneal poly(I:C) application. Furthermore, due to the possible influence of the physiology of the

pregnant dam and thus the potential influence of the results, the intravenous application was not chosen either, in order to avoid the administration of isoflurane for anesthesia, as this would represent another treatment in dams that would have to be taken into account. HMW poly(I:C) was administered at a dose of 10 mg/kg body weight (stock solution 5 mg/ml) and LMW poly(I:C) was administered at a dose of 10 mg/kg (stock solution 15 mg/ml). Immediately after the treatment, the dams were placed back to their home cages and left undisturbed. The core temperature of treated dams was scanned by the handheld reader (UID, URH-1HP) after 90 minutes and then again, every 15 minutes for another 220 minutes. The handheld reader scanned the targeted microchip information from the distance, the rats were therefore minimally disturbed during this process.

4.3 Postnatal Manipulations and Weaning

In the period between the treatment and birth, the dams were left undisturbed. Three days before the estimated birth, the dams were housed individually and provided nestlets for nest building. Out of 17 potential dams that received treatment, nine dams successfully delivered pups. The day of birth was referred to as postnatal day (PD) 0 because most dams delivered pups during the late afternoon/evening. The dams with newborn pups were left undisturbed until postnatal day (PD) 4. On PD 4, the pups were weighed, and the litter sizes were limited to 6 pups to control for variability in developmental rates (Enes-Marques & Giusti-Paiva, 2018). Excess pups were sacrificed by isoflurane (CHEMICAL IBÉRICA PV, S.L., Salamanca, Spain) inhalation with subsequent decapitation. The remaining pups (3 male and 3 female pups of each dam; N = 27 male pups and N = 27 female pups in total) underwent a battery of behavioral tests at PD 12 to assess their early neurodevelopment and social behavior. The pups were then weighed again on PD 12. Following behavioral tests, pups were left undisturbed until weaning and another weighing at PD 21. The siblings were then housed together but separated by gender in the same animal room as older pups and dams.

4.4 Early Postnatal Assessments of Neurodevelopment and Social Behavior

Juvenile behavioral tests were conducted with 54 pups in total (N = 27 females and N = 27 males). The tests were executed within one day (at around 11 am). Before the start of the

behavioral testing, the pups and their mothers were placed in the experimental room where the tests took place and were habituated in their home cages to the environment for 15 minutes. The dams were individually separated from their pups just before the beginning of the tests and placed in a different cage at a sufficient distance from the pups. They were returned to their home cage and their pups immediately after the end of the tests.

Weight Monitoring of Pups

On PD 4, 12, and 21 each pup was separated from their mothers and the rest of the litter and weighed to monitor developmental changes between treatment groups. Each pup was placed on a laboratory scale and its weight was recorded.

Ultrasonic Vocalization Recording (PD 12)

Each pup was taken from the nest and placed into a small polystyrene box (13 x 11,5 x 15 cm) with a microphone built into the lid (Ultramic 250 k microphone; 250 kHz range and sampling rate; Dodotronic, Italy) to assess social communication in pups (see Fig. 8) by recording and measuring USV calls (above 20kHz) when isolated from mother based on previous studies (Schwartzer et al., 2013; Vojtechova et al., 2021; Gzieło et al., 2023). The microphone was connected to a computer with the Audacity freeware (Audacity®; https://audacityteam.org/) used for sound recording and subsequent analysis. The emission of vocal calls was then recorded for 70 s including the initial 10 s period designated for habituation of the pup to the box. 2 parameters of the ultrasonic vocalization were analyzed: the number of USVs and average duration of a USV call (s). 2 software programs were used for the analysis: USVSEG automatic (Tachibana et al., 2020; https://github.com/rtachi-lab/usvseg), and followingly Audacity (manual; control and corrections).

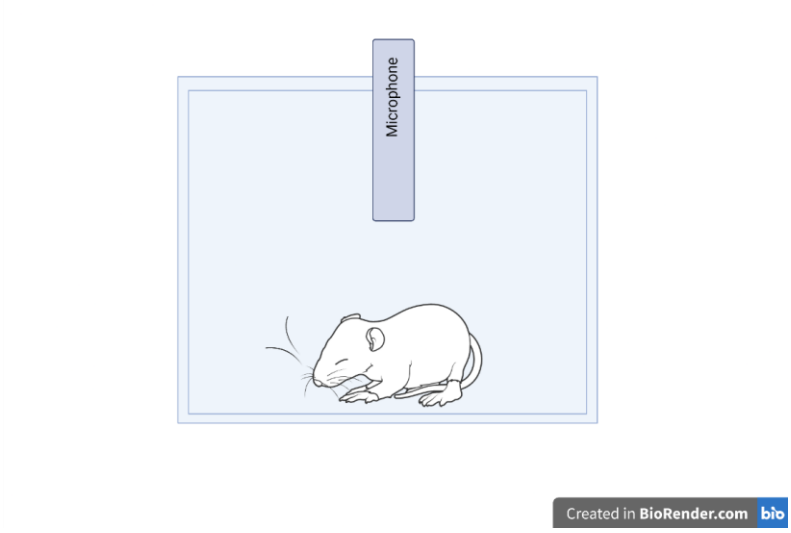

Fig. 8: Ultrasonic Vocalization test

Homing Test (PD 12)

The homing test was executed to evaluate the tendency and reflexes of pups to recognize and return to the nest of their mother and siblings, based on the previous study of Fischer et al. (2016). Each pup was individually placed into a polystyrene box (23,5 x 19,5 cm) visually divided into two halves (see Fig. 9). One half of the box contained clean bedding and the other contained bedding taken from the home cage. The pup was placed in the middle of the clean bedding section facing the wall of the box with its back turned to the center of the box. Immediately after the pup's placement in the box, the time it took the pup to turn around and cross over to the home cage bedding section was measured. The time during which the animal climbed over the line between home cage bedding and clean bedding with the rear part of the body was evaluated. This testing process was repeated three times for each pup and averaged.

Negative Geotaxis (PD12)

The pups were tested to access behavioral responses and directional movements against gravitational cues to evaluate their development of proprioceptive and sensory functions (Arsenault et al., 2014). Every pup was individually placed on an inclined (25°) platform with a non-slip surface (12 x 21 x 24 cm) facing downwards (see Fig. 9). The time it took for the pup to completely turn around 180° to face upwards was measured and recorded. This testing process was repeated three times and averaged for each pup.

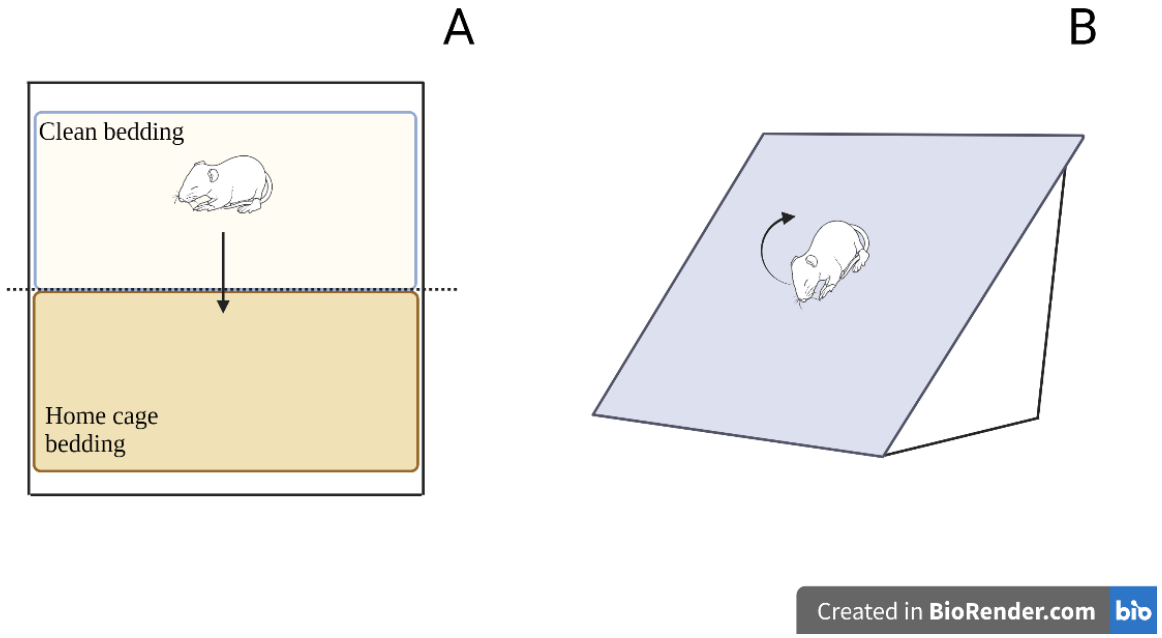

Fig. 9: Early postnatal behavioral tests. (A) Homing test; (B) Negative geotaxis

4.5 Behavioral Assessment in Adulthood

At 3 months rats underwent a battery of behavioral tests. Each day, the males were tested first, in order not to distort their behavior due to the olfactory traces left by females. Behavioral tests were timed so that anxiety-sensitive tests (such as the Elevated Plus Maze test and Open Field) were conducted first. Always, only one daily behavioral test or a part of the test was performed. The interval between tests was 0 - 2 days. Each behavioral test was performed with the animals at a similar time (around 10:00 a.m.). Before the start of each behavioral test, the rats were placed in the experimental room where the test was to take place and habituated in their home cages to the environment for 15 minutes.

Elevated Plus Maze (3mo)

Adult offspring were tested in an elevated Plus Maze (130 cm above the floor) to assess their anxiety and fear behavior (see Fig. 11). The test was carried out based on the modified work protocols of Li et al. (2021) and Su et al. (2022). The maze consisted of two opposing open arms (50 x 10 cm) and two opposing closed arms (5 x 10 cm) surrounded by 25 cm-high walls. Each experiment began by placing a rat in the center of the arena facing an open arm (same

arm for every rat); the rat was then left to explore the apparatus freely for five minutes. The experimenter was present in the room throughout the test. The apparatus was cleaned before and after each animal using water and denatured alcohol. The movement and behavior of animals were recorded by the camera mounted above the apparatus and later semi-automatically analyzed using Ethovision XT (Noldus Information Technology BV; https://www.noldus.com/ethovision-xt). The estrous cycle of the females was determined by vaginal smears after the end of the test. The frequency of open-arms visits and the time spent in opened-arms of the arena were analyzed to determine increased anxiety and explorative behavior.

Open Field Test (3mo)

The open field test was carried out to assess basal locomotion activity, explorative behavior, and anxiety in rats based on a previous study (Li et al., 2021; Su et al., 2022). The rats were placed again into a corner of an empty square arena (70 x 70 cm) with high non-transparent walls (40 cm) and were left to explore the arena freely for 15 minutes. The whole session was recorded using a camera mounted above the arena. The arena was cleaned after each animal using water and denatured alcohol. The cumulative time spent in the center (50% of the inner surface) as well as in the corners of the arena and the distance traveled was later analyzed semi-automatically using Ethovision XT (Noldus Information Technology BV; https://www.noldus.com/ethovision-xt). The results of the open field test were used also as a habituation phase and a covariate in SPSS for the ketamine-induced hyperlocomotion test.

Social Interaction Test (3mo)

Social Interaction test was used to assess the social behavior of adult offspring based on previous studies (Vojtechova et al., 2021; Gzieło et al., 2023). Each animal was exposed to the social interaction test only once. Two rats with the same sex and same treatment but from a different dam, which were never exposed to each other previously, were placed together in the opposite corners of an empty square-shaped open-field arena (70 x 70 cm) with high non-transparent walls (40 cm). Both animals were left undisturbed to explore the open field arena (see Fig. 10) and each other for 10 minutes. The whole session was recorded using a camera mounted above the arena. The arena was cleaned after each pair of animals using water and

denatured alcohol. The total time the animals spent by anogenital sniffing of the partner, non-anogenital sniffing, following the other partner, or climbing over the back of the other partner, was analyzed using Boris software (Olivier Friard, Marco Gamba; http://www.boris.unito.it/), analyzing simultaneously both animals in each pair. The estrous cycle stage of the females was determined by vaginal cytology from the vaginal smears after the end of the test session and used as a covariate in SPSS.

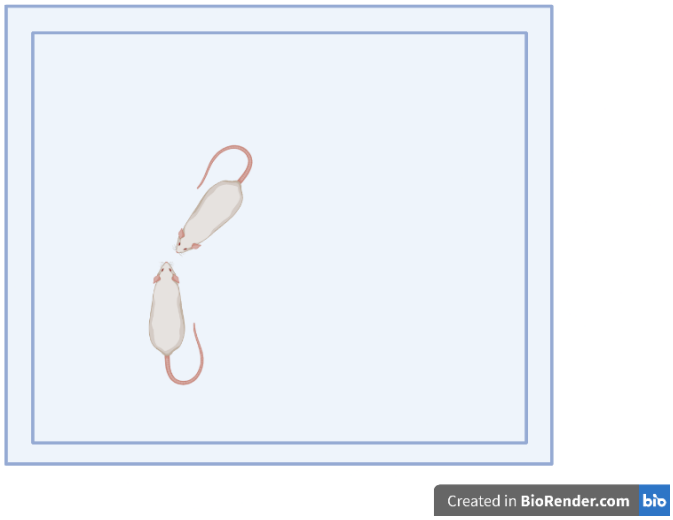

Fig. 10: Social Interction test in the Open Field

T-maze Rewarded Alternation Test (3mo) – Working Memory Task

Adult offspring underwent habituation, training, and testing in T-maze (modified protocol by Deacon & Rawlins (2006) to evaluate their working memory. The T-maze consisted of a wide base arm (15 x 50.5 cm) perpendicularly separating two opposing closed arms (side arms; 9 x 50 cm) surrounded by 28.5 cm high walls. The day before the first habituation session, animals were acclimatized to a reward stimulus (oat flakes) in their home cage. The food restriction regimen began on the day of the first habituation and lasted until the end of the experiment. The habituation phase lasted two days and served to familiarize animals with the apparatus. During the habituation phase, both side arms were open and contained four oat flakes in glass wells located at their ends. Animals were individually placed in the base arm of the apparatus and left to explore the apparatus freely. Habituation sessions lasted ten minutes; the number of consumed oat flakes was recorded at the end of each session. Rats proceeded to the training phase on the condition that they consumed the food reward during the second habituation

session within the time limit of three minutes. Next, animals were trained to actively search for and consume the food reward (three oat flakes in glass wells) located in one randomly chosen side arm of the apparatus (training phase) during three training sessions. Access to the other arm was blocked by a removable divider. Each training day consisted of six trials: three trials forced the animal to enter the left arm and three forced the animal to enter the right arm; the order of the rewarded arms was chosen randomly. The training trial was terminated if the animal failed to consume the reward from the forced arm within two and a half minutes. Animals proceeded to the test phase when they consumed the reward within three minutes in 50 % of training trials on the last day. The testing phase took place over two days and consisted of eight trials per day (four forced and four choice trials; side arms to be blocked by the divider were chosen randomly). A choice trial always followed a forced trial with a delay of one minute during which the animal stayed in its home cage (see Fig. 11). Entry to the arm previously blocked by the divider during the forced choice was considered a correct choice (i.e., alternation). The experimenter was present in the room throughout the whole experiment. The apparatus was cleaned using water and denatured alcohol before and after each animal and in the delay between forced and choice trials during the test trials to minimize odor cues. All habituation, training, and testing sessions were recorded using a camera mounted above the apparatus. Based on the number of correct alternations in eight choice test trials, the percentage of alternation rate for each animal was evaluated and analyzed. Success in learning in the training phase was also analyzed, i.e., how many of the 18 trials were successfully completed (reward consumed within the time limit).

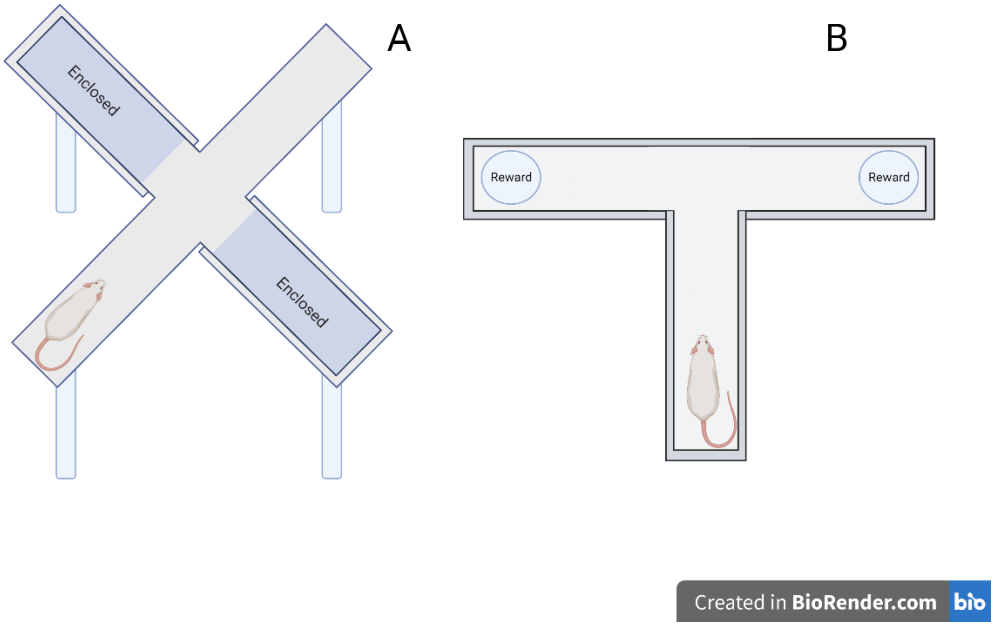

Fig. 11: Behavioral test in adulthood. (A) Elevated Plus Maze, (B) T-maze Rewarded Alternation Test

Ketamine-Induced Hyperlocomotion in the Open Field test (3mo)

The ketamine-induced hyperlocomotion test was conducted to access schizophrenia-like behavior in rats. Ketamine is a non-competitive NMDA-receptor antagonist, therefore has been used as a replacement of the MK-801 treatment, which was already conducted by previous studies focusing on schizophrenia-like behavior of poly(I:C) offspring (Zuckerman & Weiner, 2005; Zavitsanou et al., 2014). The rats were randomly assigned to a single injection of a subanesthetic dose of ketamine (5 mg/kg) or an injection of endotoxin-free vehicle solution (0,9% NaCl; Fresenius Kabi, s.r.o., Na Strži, Praha 4). Solutions were prepared on the day of the treatment: ketamine solution (25 mg/ml) was prepared by diluting stock solution (50 mg/ml; Bioveta a.s.; Komenského, Ivanovice na Hané) with vehicle solution. Rats received the treatment by subcutaneous injection into their lower back. Immediately after the treatment, rats were individually placed into the corner of the same open field arena (70 x 70 cm) with high non-transparent walls (40 cm) and their movement in the apparatus was recorded for 15 minutes. The open field was cleaned before and after each animal using water and denatured

alcohol. The distance traveled after the treatment in the arena was later analyzed semi-automatically using Ethovision XT (Noldus Information Technology BV; https://www.noldus.com/ethovision-xt) and was compared to their basal locomotion assessed in the Open Field test.

4.6 Statistical Analysis and Data Visualization

Data from behavioral tests and experiments were analyzed on IBM SPSS Statistics version 26 (New Orchard Road, Armonk, New York); effect sizes were calculated based on mean difference and calculated using online software Estimation Stats (Ho et al., 2019; https://www.estimationstats.com/#/).

The level of accepted statistical significance was set at $p < 0.05$. Graphs were created using Estimation Stats (Ho et al., 2019; https://www.estimationstats.com/#/) and Microsoft Excel (Microsoft 365). All early postnatal tests (PD 4, 12, 21), as well as tests in adulthood (3 m.o.), were analyzed using one-way ANOVA or two-way ANOVA, or ANCOVA (if a covariate was analyzed). The pregnancy success rate was analyzed by the Chi-Square test. Stages of the estrous cycle were used as covariates in the case of the Social Interaction test, given that in this test the cycle stages could affect the results. In other tests, the estrous cycle effect was not taken into account, based on a meta-analysis of Becker et al. (2016) that showed that the estrous cycle phase had no behavioral effect in these tasks. In the case of a significant main effect in ANOVA, the Tukey HSD post hoc test was used. In the case of a significant main effect in ANCOVA, the Bonferroni test was used for multiple comparisons adjustment based on estimated marginal means. For the correlation analysis, Pearson's Correlaton analysis was used. The results from the data are presented as the means ± standard errors of the mean (SEM).

5 Results

5.1 Verification of MIA and Pregnancy Outcomes

Core Body Temperature Changes

The maternal immune response after poly(I:C) administration was verified by analyzing core body temperature measurements in 9 pregnant dams (N = 9) that later successfully delivered pups (Fig. 12). After 90 minutes from the poly(I:C) treatment, a significant difference in the core body temperature was observed [ANOVA, treatment: $F(2,6) = 7.843$; $p = 0.021$]. *Post hoc* test showed elevated body temperature in HMW females compared to LMW females (Tukey, $p = 0.019$). Another difference in body temperatures was found at 150 minutes [ANOVA, treatment: $F(2,6) = 9.189$; $p = 0.015$]. Follow-up analysis showed that the body temperature significantly increased in the HMW dams compared to the LMW dams ($p = 0.019$), and also compared to the control dams (Tukey, $p = 0.029$). A significant change in body temperature was again found at 195 minutes [ANOVA, treatment: $F(2.6) = 5.151$; $p = 0.050$] in the HMW dams compared to control dams (Tukey, $p = 0.043$). Next, significantly different body temperature was observed at 210 minutes [ANOVA, treatment: $F(2,6) = 11.411$; $p = 0.009$]. The body temperature of the HMW dams was significantly elevated compared to the LMW dams (Tukey, $p = 0.017$), and compared to the control dams (Tukey, $p = 0.013$).

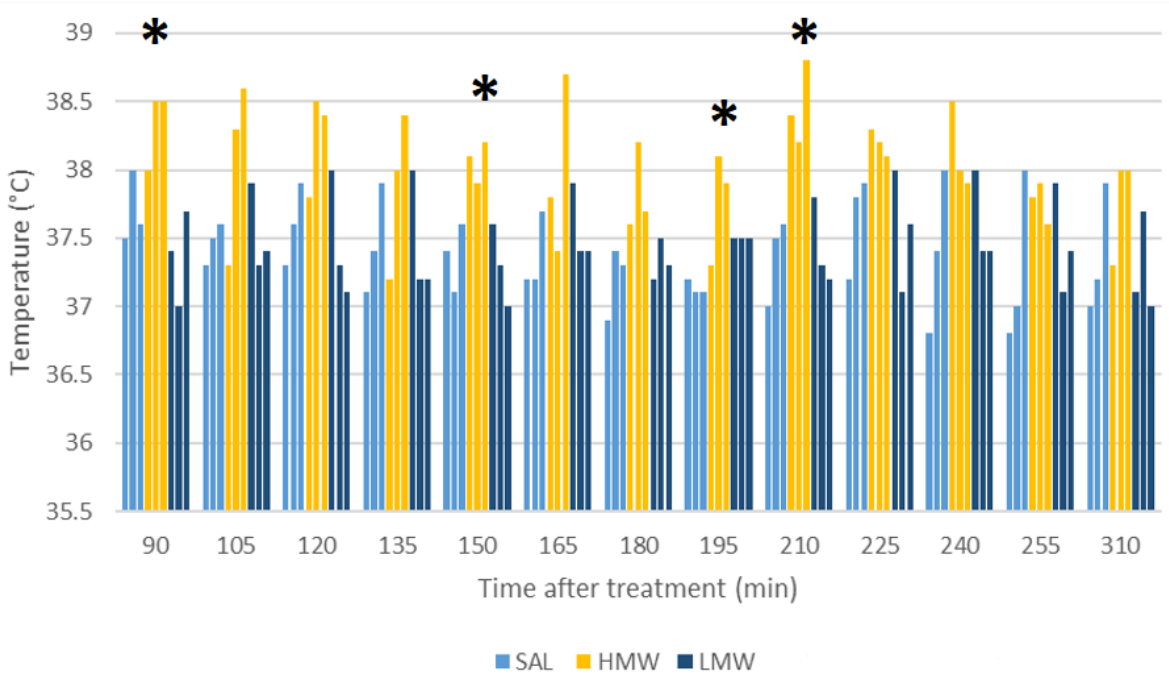

Fig. 12: Core body temperature changes in pregnant dams within 310 minutes after the administration of poly(I:C) on GD 14. The core body temperature was measured after 90 minutes after the treatment and then every 15 minutes until reaching the time point of 310 minutes after treatment. Every column represents a pregnant female and her temperature at a specific time point after treatment. The colors show which treatment the females underwent, N = 3 per treatment group. *p < 0.01 compared to LMW, SAL, or both treatment groups.

Pregnancy outcomes

Out of 17 dams that received treatment, nine dams successfully delivered pups. The effect of the poly(I:C) treatment on the successful delivery of pups and possible litter abortion was analyzed (Fig. 13). A Chi-Square test of independence was performed to evaluate the relationship between the treatment of the dams and the pregnancy success rate. The Chi-Square test showed no significant difference between treatments [$X^2(2, N = 17) = 0.49$; $p = 0.784$].

Fig. 13: The pregnancy success rate of treated dams. Within the nine successful dams, there were three dams in every treatment group [HMW, N = 3; LMW, N = 3; SAL, N = 3]. Unsuccessful pregnancies included eight dams in total [HMW, N = 2; LMW, N = 2; SAL, N = 4]. No significant results were found $p > 0.05$.

Next, the effect of the molecular weight of poly(I:C) on the litter size was analyzed. The poly(I:C) treatment had no significant effect on the size of the litter [ANOVA, treatment: $F(2,6) = 0.750$; $p = 0.512$], Fig. 14.

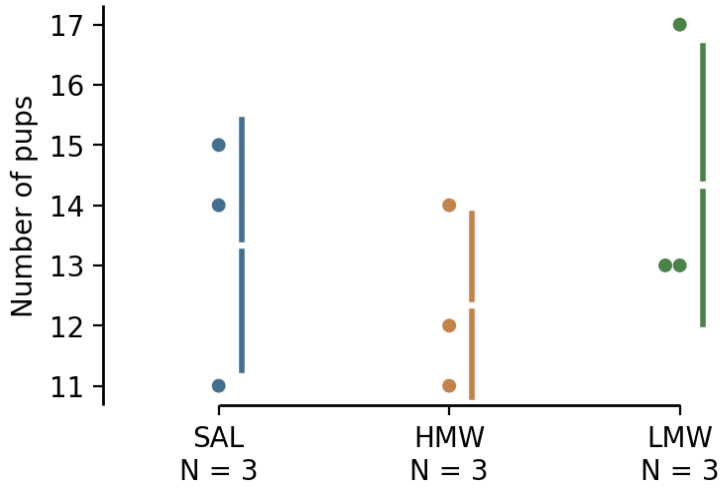

Fig. 14: Litter size. N = 3 per treatment group. The results are presented as the means ± standard errors of the mean (SEM). No significant results were found $p > 0.05$.

5.2 Poly(I:C)-Induced MIA Effects on Weight Development and Early-Postnatal Behavior of Offspring

The early postnatal behavioral tests including weighing of the pups were conducted with 54 pups in total (N = 27 females; N = 27 males) of which 18 pups were in every treatment group (N = 9 females; N = 9 males). The pups were weighed on PD 4, 12, and 21. The order of developmental tests was always maintained as follows: Ultrasonic Vocalization test, Negative Geotaxis, Homing test.

Weight Changes of Pups

Lower body weight was observed in LMW compared to HMW pups. Specifically, within the poly(I:C) groups there was a significant body-weight difference on PD4 between groups [ANOVA: $F(2, 51) = 7.712$; $p = 0.001$]. The *Post hoc* test showed that this was due to the lower body weight of LMW pups compared to HMW pups (Tukey, $p = 0.01$) (Fig. 15). There was no difference in body weight between either HMW or LMW and control pups (both $p > 0.005$). As the sex of the pups had not been determined on PD 4, the difference between the sexes was not analyzed. However, following Pearson's correlation analysis to determine the relationship between the body weight of pups on PD 4 and the size of the litter from which the pups came showed a negative correlation between the body weight and the litter size, which was statistically significant ($r = -0.740$, $n = 54$, $p < 0.001$).

The lower body-weight differences were also significant on PD12 (Fig. 15) [two-way ANOVA; treatment: $F(2, 48) = 4.127$; $p = 0.022$].The *Post hoc* test showed a significantly lower-body weight of LMW pups compared to HMW pups (Tukey, $p = 0.022$). There was no significant effect of sex [two-way ANOVA: $F(1,48) = 0.969$; $p > 0.05$] or sex-treatment interaction [two-way ANOVA: $F(2,48) = 0.003$; $p > 0.05$]. However, following Pearson's correlation analysis to determine the relationship between the body weight of pups on PD 12 and the size of the litter from which the pups came showed a negative correlation between the body weight and the litter size, which was statistically significant ($r = -0.380$, $n = 54$, $p = 0.005$).

On PD21 there were no significant differences between groups [two-way ANOVA; treatment: $F(2,48) = 1.288$; $p > 0.05$], (Fig. 16). There was no significant effect of sex [two-way ANOVA: $F(1,48) = 0.575$; $p > 0.05$], or sex-treatment interaction [two-way ANOVA: $F(2,48) = 0.616$; $p > 0.05$]. In addition, following Pearson's correlation analysis to determine the relationship between the body weight of pups on PD 21 and the size of the litter from which

the pups came showed a negative correlation between the body weight and the litter size, which was statistically significant (r = -0.431, n = 54, p = 0.001).

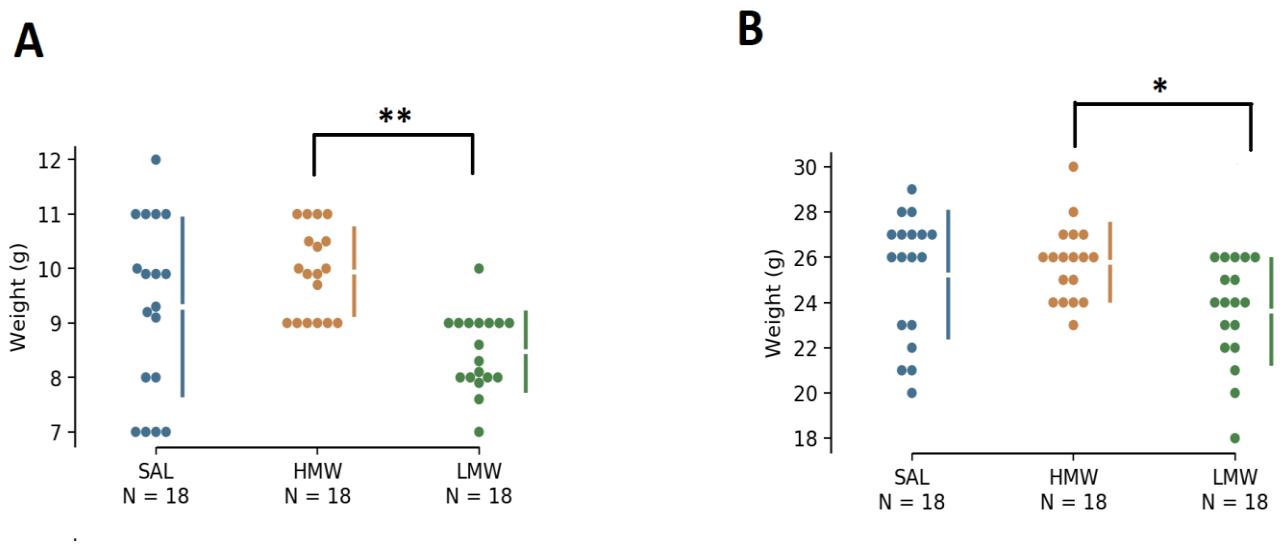

Fig. 15: The weight of pups on PD 4 and PD 12. (A) PD4. (B) PD12. N = 18 per treatment group. The results are presented as the means ± standard errors of the mean (SEM). *p < 0.05; **p < 0.01.

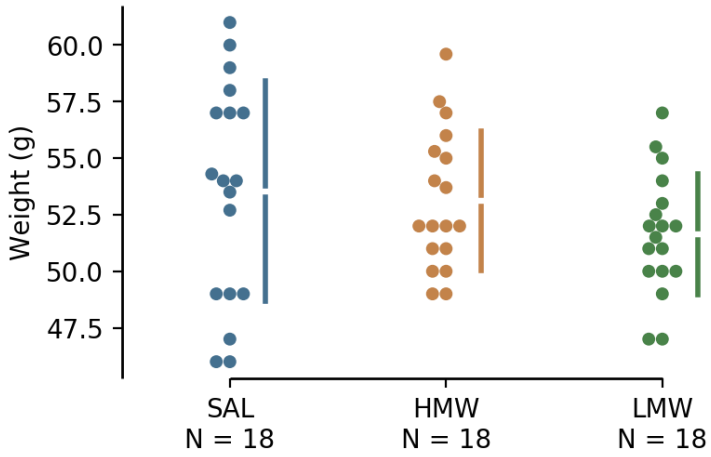

Fig. 16: The weight of pups on PD 21. N = 18 per treatment group. The results are presented as the means ± standard errors of the mean (SEM). *p < 0.01.

Ultrasonic Vocalization

Prenatal poly(I:C) exposure did not significantly affect the number of USVs calls emitted by the pups, [two-way ANOVA; treatment: $F(2, 48) = 1.996$; $p = 0.147$], (Fig. 17). There was no significant effect of sex [two-way ANOVA; $F(1,48) = 0.276$; $p > 0.05$], or sex-treatment interaction [two-way ANOVA; $F(2,48) = 0.035$; $p > 0.05$].

Poly(I:C) did not significantly affect the average duration of USVs of pups in LMW or HMW group [two-way ANOVA; treatment: $F(2,48) = 1.495$; $p > 0.05$], (Fig. 17). There was no significant effect of sex [two-way ANOVA; $F(1,48) = 1.571$; $p > 0.05$], or sex-treatment effect [two-way ANOVA; $F(2,48) = 0.187$; $p > 0.05$].

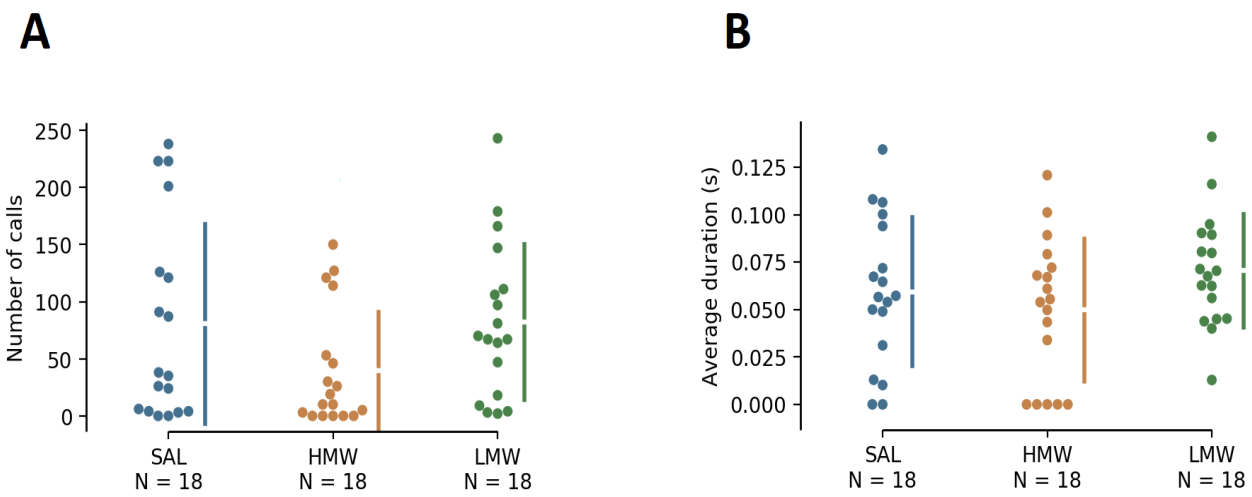

Fig. 17: The frequency and average duration of USV calls of pups in the Ultrasonic Vocalization test. (A) Frequency of USV calls. (B) Average duration of USV calls. N = 18 per treatment group. The results are presented as the means ± standard errors of the mean (SEM). *$p < 0.01$ in comparison to the control (SAL) group.

Negative Geotaxis

Prenatal poly(I:C) exposure affected males and females differently [two-way ANOVA; sex-treatment interaction: $F(2,48) = 3.429$; $p = 0.041$]. To dissect the interaction further the data were split by sex and analyzed using one-way ANOVA. There were no significant effects in males; in females, there was a significant effect of treatment [ANOVA: $F(2,24) = 4.656$;

p = 0.020], (Fig. 18). The *Post hoc* test showed differences in females between treatments showed significantly increased average time in females in the LMW treatment group compared to the control group (Tukey; p = 0.017). The differences between the average time to turn in males were not significant between treatment groups (p > 0.05).

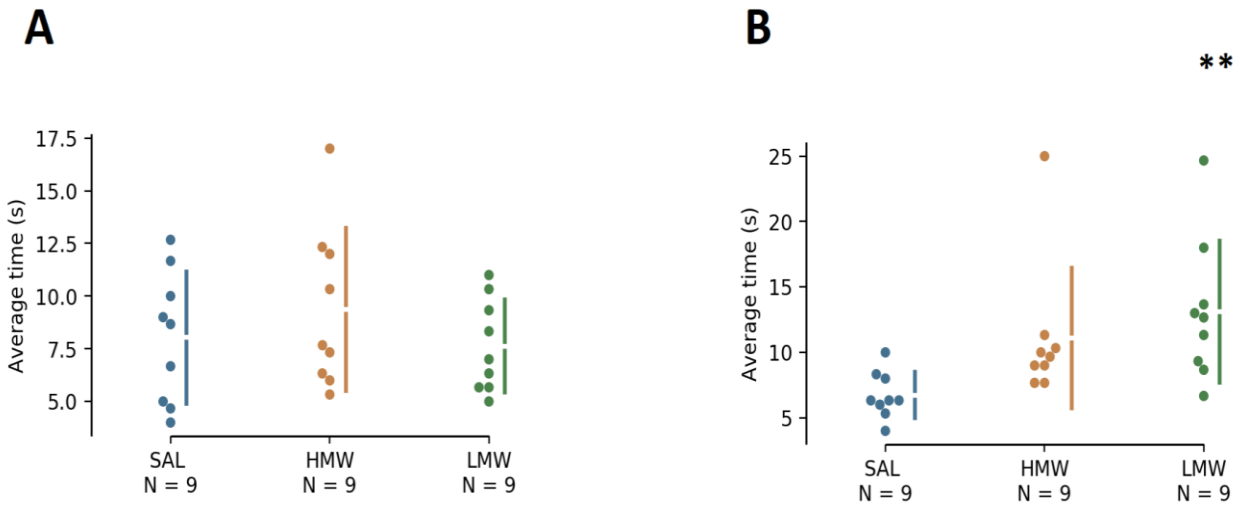

Fig. 18: Average time in the Negative Geotaxis test. (A) males. (B) females. N = 9 per treatment group. The results are presented as the means ± standard errors of the mean (SEM). **p < 0.01 in comparison to the control (SAL) group.

Homing Test

Prenatal poly(I:C) exposure significantly changed the average times it took different groups to cross the division line to the home cage bedding [two-way ANOVA; treatment: F(2, 47) = 3.273; p = 0.047], (Fig. 19). The *Post hoc* test showed that HMW-treated pups were slower compared to the control group (Tukey, p = 0.033). There was no significant effect of sex [F(1,47) = 0.001; p > 0.05], or sex-treatment effect [F(2,47) = 0.554; p > 0.05].

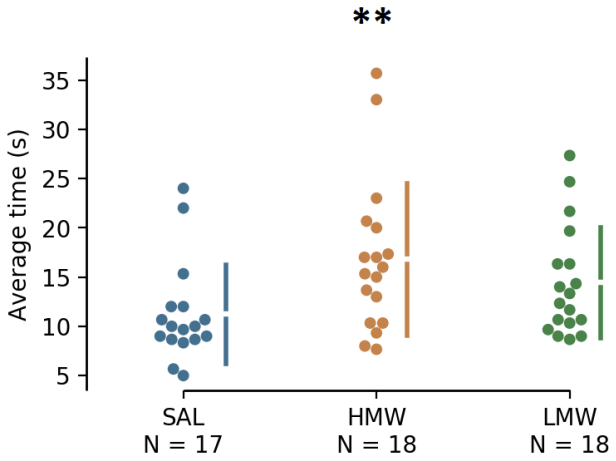

Fig. 19: Average time in the Homing test. N = 18 per LMW and HMW treatment group; N = 17 in SAL treatment group. One female from the saline group failed to transfer the division line during all three trials in the Homing test. The results are presented as the means ± standard errors of the mean (SEM). **p < 0.01 in comparison to the control (SAL) group.

5.3 Poly(I:C)-Induced MIA Effects on Behavior of Adult Offspring

The behavioral tests in adulthood were conducted with 54 rats in total (N = 27 females; N = 27 males) of which 18 rats were in every treatment group (N = 9 females; N = 9 males). One female from the LMW group was excluded from the Elevated Plus Maze due to repeated falling from the open arm of the apparatus.

Elevated Plus Maze (EPM)

In the EPM test the duration of time spend in the open arms and the number of entries into the open arms were analyzed and considered as a measure of low anxiety.

There was a significant difference between groups [two-way ANOVA; treatment: $F(2,47) = 6.539$, $p = 0.003$]. Rats exposed to prenatal poly(I:C) spend significantly more time in open arms (Tukey, LMW compared to saline, $p = 0.003$; HMW compared to saline, $p = 0.043$), (Fig. 20). There was no significant effect of sex [two-way ANOVA: $F(1,47) = 1.821$, $p > 0.05$], or sex-treatment interaction [two-way ANOVA: $F(2,47) = 0.623$, $p > 0.05$].

The number of entries into the open arms of the EPM differed between groups [two-

way ANOVA; treatment: F(2,47) = 8.509, p = 0.001] *Post hoc* test showed that the number of entries was significantly higher in the rats exposed to prenatal poly(I:C) compared to controls (Tukey, LMW compared to controls: p = 0.002; HMWcomapred to controls: p = 0.005). There was a significant effect of sex [two-way ANOVA: F(1,47) = 5.475, p = 0.024] showing fewer entries of the males, but not a sex-treatment interaction [two-way ANOVA: F(2,47) = 0.029, p > 0.05].

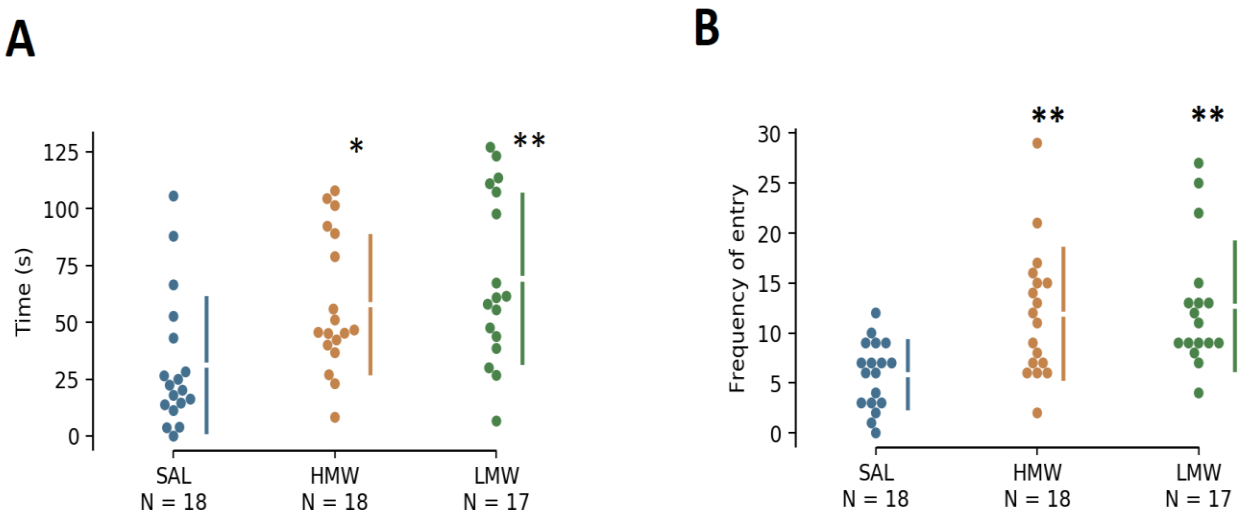

Fig. 20: Total time spent in open arms and the frequency of entry into the open arms of the Elevated Plus Maze apparatus. (A) Time spent in open arms. (B) Number of entries into the open arms. N = 18 per SAL and HMW treatment group; N = 17 in the LMW treatment group. The results are presented as the means ± standard errors of the mean (SEM).*p < 0.05, **p < 0.01 in comparison to the control (SAL) group.

Open Field Test (OF)

Next, the time spent in the center of the OF was analyzed: a measure of exploratory behavior and low anxiety. There was a significant sex-treatment interaction [two-way ANOVA, F(2,48) = 4.335; p = 0.019]. In follow-up, it was found that there was an effect of treatment only in males [one-way ANOVA; treatment: F(2,24) = 8.152; p = 0.002], (Fig. 21). The *Post hoc* test showed that only male rats prenatally exposed to MIA by LMW poly(I:C) showed a significantly higher duration of total time spent in the center of the OF compared to the control group (Tukey, p = 0.001). In females, the time spent in the center was not significantly affected

by poly(I:C) treatment ($p > 0.05$).

At the same time, the time spent in the corners of the OF was analyzed. There was a significant sex-treatment interaction [two-way ANOVA, $F(2,48) = 4.102$; $p = 0.023$], (Fig. 22). Follow-up analysis showed that the differences in total time spent in the corners of the Open Field arena were significant in males [one-way ANOVA; treatment: $F(2,24) = 4.306$; $p = 0.025$]. The *Post hoc* test showed lower total time in males in the LMW group compared to the control group (Tukey, $p = 0.020$). In females, there was no significant effect of poly(I:C) treatment ($p > 0.05$).

Next, locomotor activity in Open Field was compared between adult rats prenatally treated with poly(I:C) and controls. The sex-treatment interaction was significant [two-way ANOVA; treatment: $F(2,48) = 3.151$; $p = 0.052$]. When dissecting this interaction it was found that there was a significant effect of treatment only in males [one-way ANOVA: $F(2,24) = 5.172$; $p = 0.014$]. The *Post hoc* test revealed that Poly(I:C) significantly increased the basal locomotion only of males in the LMW group, compared to the control group (Tukey, $p = 0.015$) (Fig. 23). The basal locomotion of females in the OF was not significantly affected by poly(I:C) treatment ($p > 0.05$).

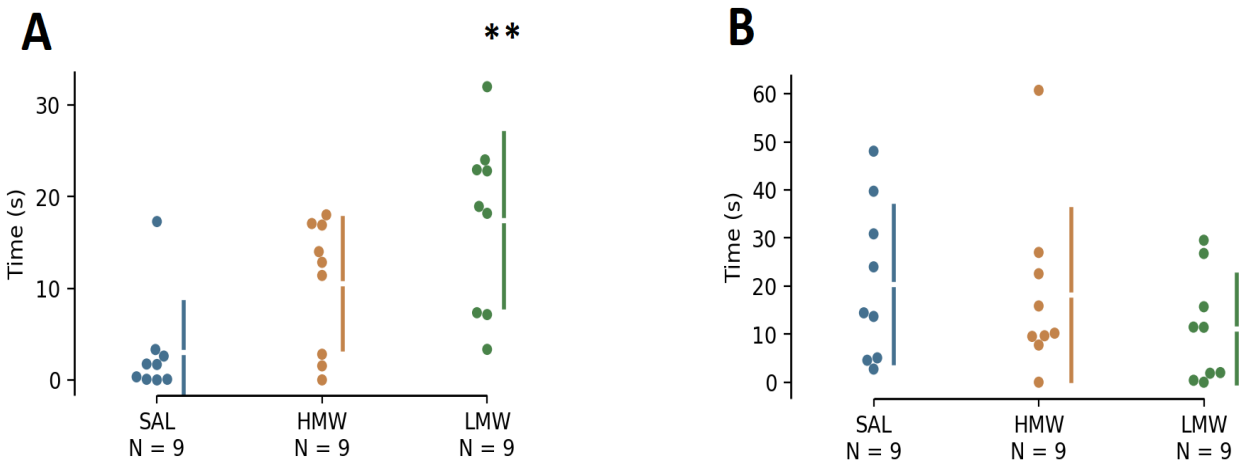

Fig. 21: Total time spent in the center of the Open Field arena. (A) males. (B) females. N = 9 per treatment group. The results are presented as the means ± standard errors of the mean (SEM). **$p < 0.01$ in comparison to the control (SAL) group.

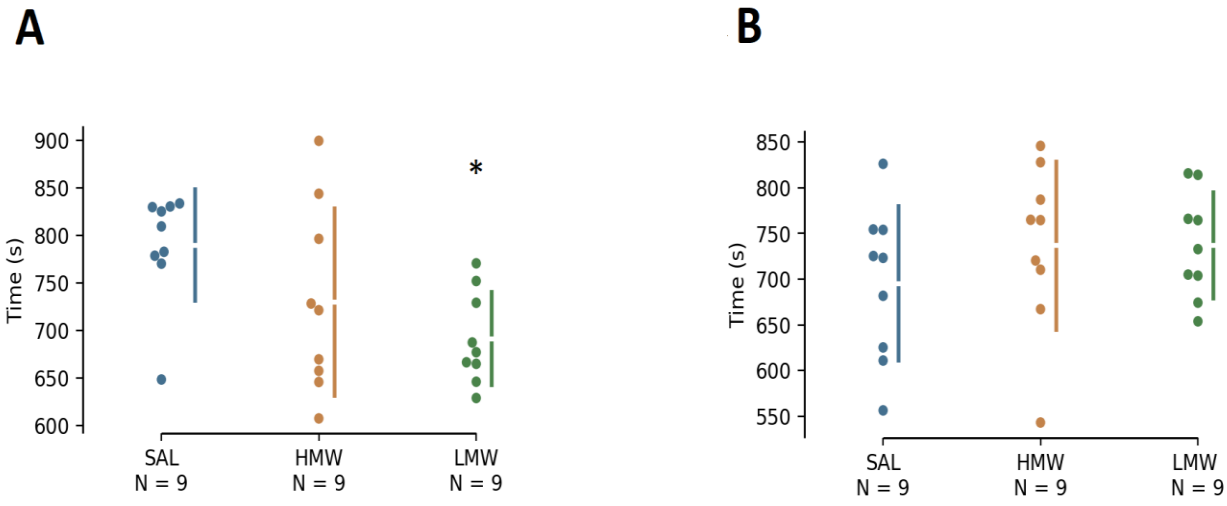

Fig. 22: Total time spent in the corners of the Open Field arena. (A) males. (B) females. N = 9 per treatment group. The results are presented as the means ± standard errors of the mean (SEM). *p < 0.05 in comparison to the control (SAL) group.

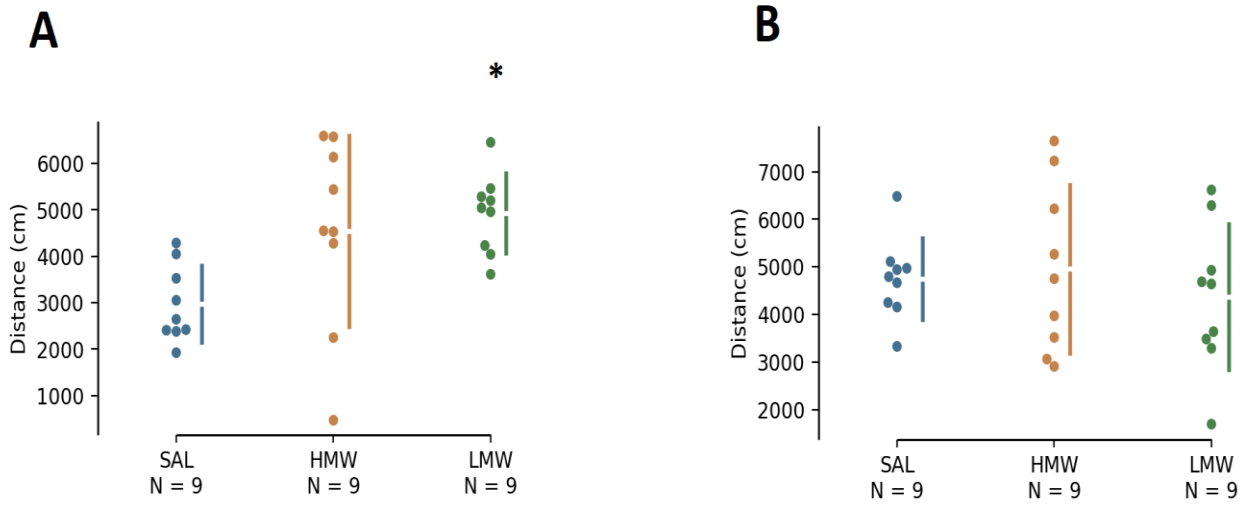

Fig. 23: The duration of the basal locomotion in the Open Field arena. (A) males. (B) females. N = 9 per treatment group. The results are presented as the means ± standard errors of the mean (SEM). *p < 0.05 in comparison to the control (SAL) group.

Social interaction test

During the social interaction test, total durations of anogenital sniffing, non-anogenital sniffing, following behaviors, and climbs were analyzed.

Poly(I:C)-induced MIA significantly affected anogenital sniffing social interaction. There was a significant sex-treatment interaction [two-way ANCOVA: $F(2,47) = 6.142$; $p = 0.004$]. There was no significant effect of the estrous cycle [two-way ANCOVA: $F(1,47) = 1.303$; $p > 0.05$]. Follow up test showed that there was a difference in treatment in males [one-way ANOVA: $F(2,25) = 4.784$; $p = 0.018$] and also in females [one-way ANOVA: $F(2,25) = 4.188$; $p = 0.028$], indicating that poly(I:C) affected anogenital sniffing differently in males than females. The *Post hoc* test showed that the total time of anogenital sniffing behavior was lower in males (Fig. 24) for both HMW and LMW groups compared to controls (Tukey, HMW vs. controls: $p = 0.040$; LMW vs. controls: $p = 0.029$). In females, poly(I:C) groups did not differ from the controls, but the LMW group showed significantly more anogenital sniffing compared to the HMW group (Tukey, $p = 0.029$).

Poly(I:C) did not significantly affect non-anogenital sniffing behavior compared to the control group (Fig. 25), [two-way ANCOVA; treatment: $F(2,47) = 2.085$; $p > 0.05$]. The effect of sex [two-way ANCOVA: $F(1,47) = 1.736$; $p > 0.05$], and sex-treatment interaction [two-way ANCOVA: $F(2,47) = 1.326$; $p > 0.05$] was not significant. The effect of the estrous cycle was also not significant [two-way ANCOVA: $F(1,47) = 1.220$; $p > 0.05$].

Next, Poly(I:C) did not significantly affect the following behavior compared to the control group [two-way ANCOVA; treatment: $F(2,47) = 2.338$; $p > 0.05$], (Fig. 25). The effect of sex [two-way ANCOVA: $F(1,47) = 4.855$; $p > 0.05$], and sex-treatment interaction [two-way ANCOVA: $F(2,47) = 2.480$; $p > 0.05$] was not significant. The effect of the estrous cycle was not significant, [two-way ANCOVA: $F(1,47) = 2.304$; $p > 0.05$].

The number of climbs was also not significantly affected after MIA by poly(I:C) [two-way ANCOVA; treatment: $F(2,47) = 0.869$; $p > 0.05$], (Fig. 26). The effects of sex [two-way ANCOVA: $F(1,47) = 0.237$; $p > 0.05$], and sex-treatment interaction [two-way ANCOVA: $F(2,47) = 2.548$; $p > 0.05$] were not significant. The effect of the estrous cycle was not significant [two-way ANCOVA: $F(1,47) < 0.001$; $p > 0.05$].

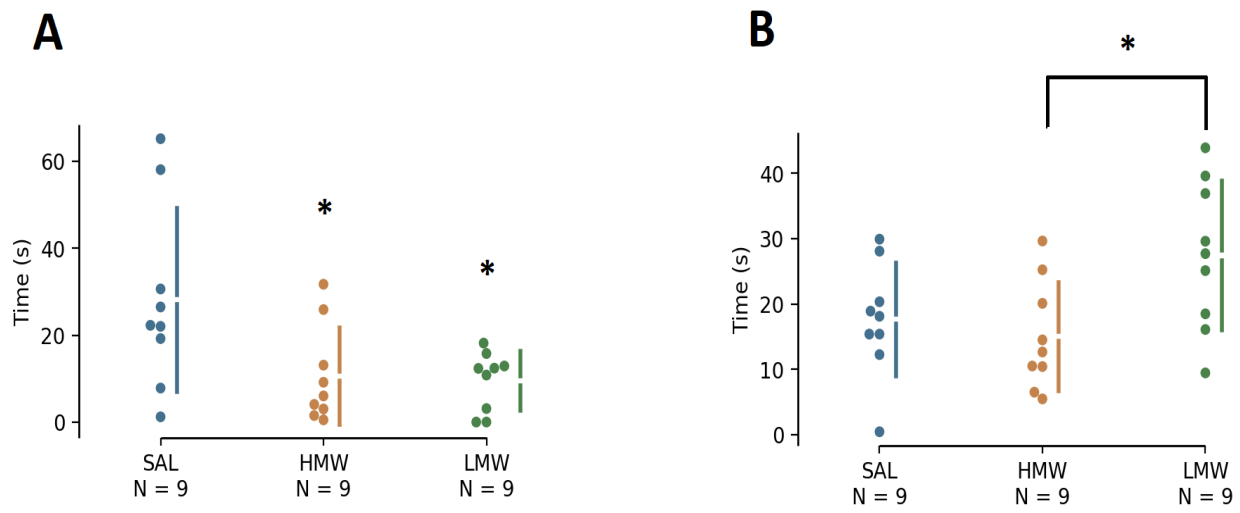

Fig. 24: Total time of anogenital sniffing behavior in the Social Interaction test. (A) males. (B) females. N = 9 per treatment group. The results are presented as the means ± standard errors of the mean (SEM). *p < 0.05.

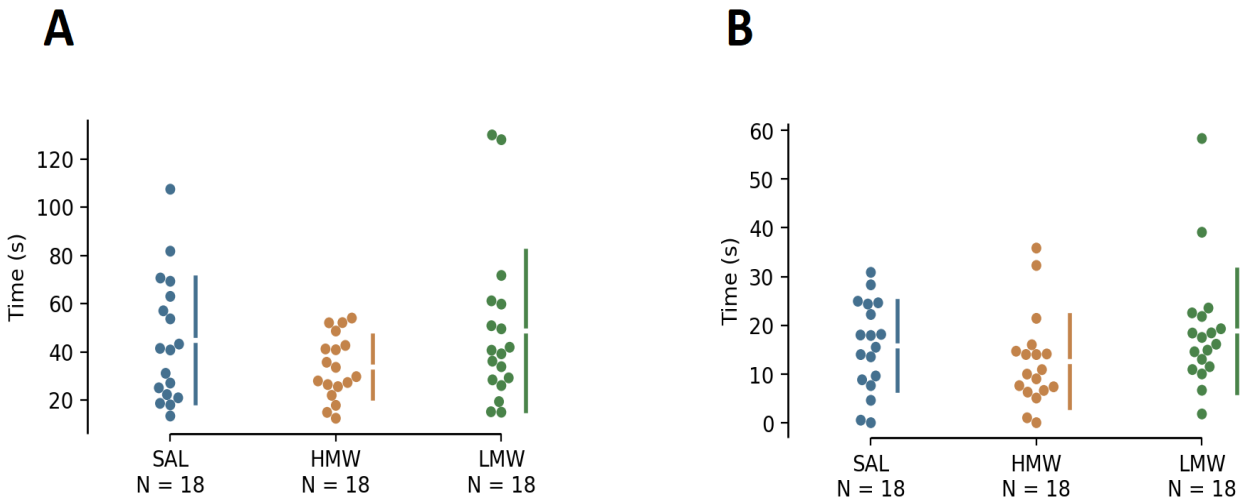

Fig. 25: Total time of the non-anogenital sniffing and following behavior in the Social Interaction test. (A) Time of non-anogenital sniffing behavior. (B) Time of the following behavior. N = 18 per treatment group. The results are presented as the means ± standard errors of the mean (SEM). No significant results p > 0.05 in comparison to the control (SAL) group.

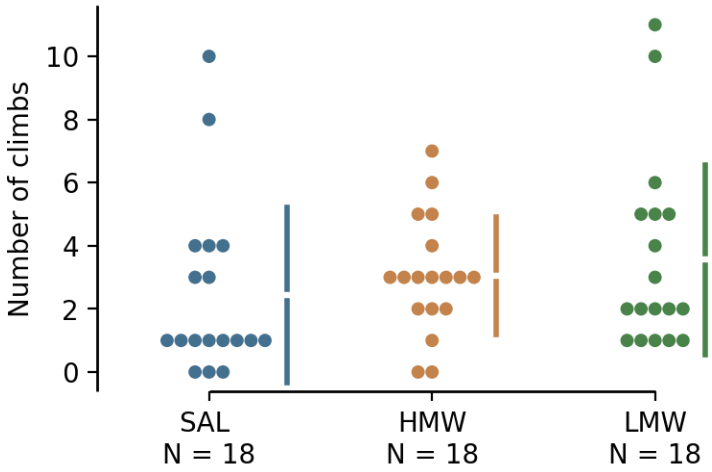

Fig. 26: Total number of climbs in the Social Interaction test. N = 18 per treatment group. The results are presented as the means ± standard errors of the mean (SEM). No significant results $p > 0.05$ in comparison to the control (SAL) group.

T-maze Rewarded Alternation Test

During the Rewarded Alternation test the percent (%) of correct alternations and successfully completed training trials were analyzed, as a measure of stereotypical behavior.

Poly(I:C)-induced MIA did not significantly affect the alternation rate compared to the control group [two-way ANOVA; treatment: $F(2,48) = 2.616$; $p > 0.05$]. There was no significant effect of sex [two-way ANOVA: $F(1,48) = 0.924$; $p > 0.05$], or sex-treatment interaction [two-way ANOVA: $F(2,48) = 0.658$; $p > 0.05$].

Next, poly(I:C) did not significantly affect successful trials in the training phase compared to the control group of offspring [two-way ANOVA; treatment: $F(2,48) = 1.852$; $p > 0.05$], (Fig. 27). There was no significant effect of sex [two-way ANOVA; treatment: $F(1,48) = 3.234$; $p > 0.05$], or of sex-treatment interaction [two-way ANOVA; treatment: $F(2,48) = 3.298$; $p > 0.05$].

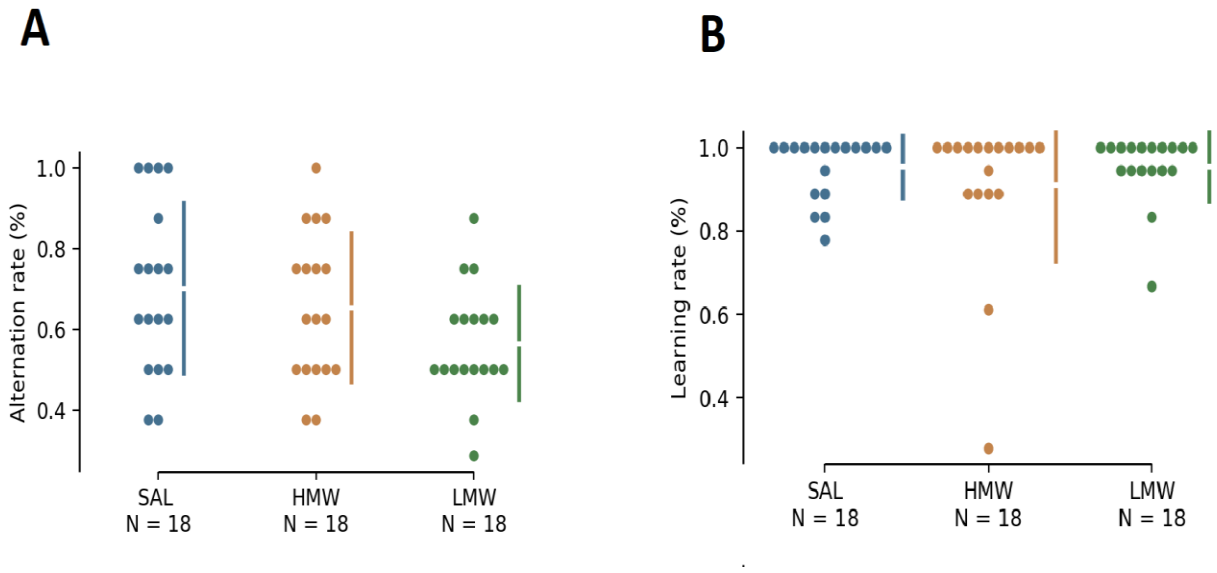

Fig. 27: T-maze Rewarded Alternation test. (A) Rate of rewarded alternations in T-maze. (B) Rate of learning. N = 18 per treatment group. The results are presented as the means ± standard errors of the mean (SEM). No significant results $p > 0.05$ in comparison to the control (SAL) group.

Ketamine-Induced Hyperlocomotion test

In the Ketamine-Induced Hyperlocomotion test, the traveled distance was analyzed to assess stimulant-induced hyperactivity.

The data were analyzed using one-way ANCOVA for each treatment group separately, as each treatment group was split into animals treated with Ketamine and animals treated with saline. The basal locomotion of each animal analyzed in the previous OF test was set as a covariate. Poly(I:C)-induced MIA did not significantly affect the locomotion after ketamine administration compared to the same-group controls [LMW, one-way ANCOVA; treatment: $F(1,13) = 0.145$; $p > 0.05$; HMW, one-way ANCOVA; treatment: $F(1,13) = 1.785$; $p > 0.05$; SAL, one-way ANCOVA; treatment: $F(1,13) = 0.784$; $p > 0.05$], (Fig. 28).

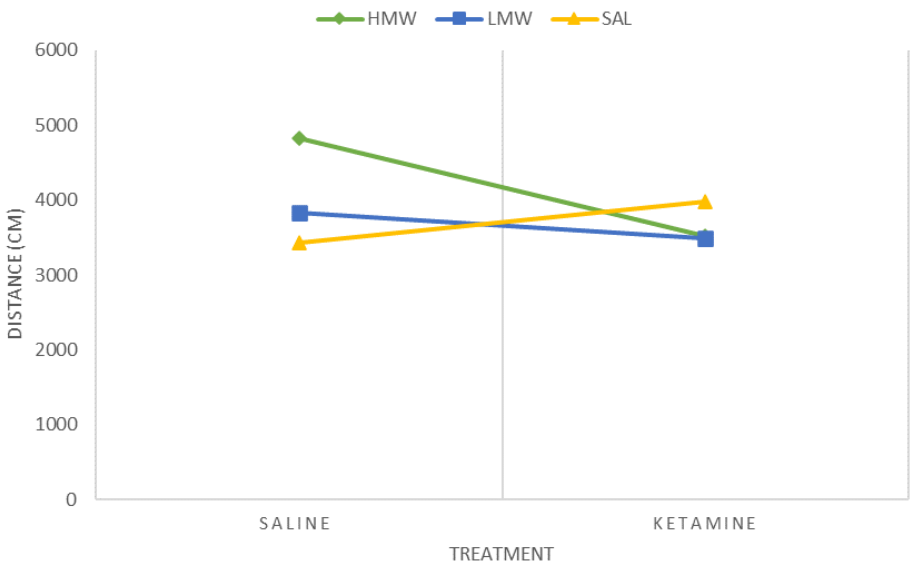

Fig. 28: Average distance of locomotion after treatment in the Ketamine Induced Hyperlocomotion test. N = 18 per treatment group. The results are presented as the means ± standard errors of the mean (SEM). No significant results $p > 0.05$.

6 Discussion

6.1 Prenatal Manipulation and Treatment

The first aim of this thesis was to induce and verify the presence of maternal immune activation in pregnant dams by HMW and LMW poly(I:C) on GD 14. Of the 17 dams that were administered with poly(I:C) or saline, 9 dams successfully delivered pups (3 females in every treatment group). In contrast with the results of Kowash et al. (2019) and Brown et al. (2022), our results did not show a significant effect of the poly(I:C) treatment on the reduced birth rate. Regardless of the treatment, one of the explanations of the reduced birth rate could be a spontaneous abortion of the litter, which can be caused by several reasons (Wiebold et al., 1986; Arck et al., 1995), false pregnancies of the females (Terkel, 1988), or incorrectly recognized pregnancy.

Our results analyzing core body temperature changes revealed significantly elevated core body temperature at several time points in dams treated by HMW poly(I:C) compared to dams treated by SAL or/and LMW dams. This possibly indicates a stronger immune response (fever induction) after HMW poly(I:C) in agreement with previous studies (Mian et al., 2013; Zhou et al., 2013, Careaga et al., 2018; Mueller et al., 2019).

In summary, the first aim was fulfilled: MIA model was induced in HMW-treated dams, as indicated in changes in core body temperature. This does not exclude MIA in LMW-treated groups, as MIA could be still present without changes in core body temperature.

6.2 Early Postnatal Development and Behavior of Poly(I:C) Offspring

The diploma thesis examined the impact of MIA induced by HMW and LMW poly(I:C) administered on GD 14 on the behavior of rat offspring in early postnatal days. The effect of HMW and LMW poly(I:C) treatments on the weight of pups on PD 4, 12, and 21 was evaluated and revealed a significantly lower body weight of pups of the LMW group compared to the HMW group on PD 4 and PD 12 regardless of sex, but not compared to the control group. This weight difference between groups was normalized by PD 21. Further analysis revealed a correlation between the body weight and the litter size of pups on both PD 4 and PD 12, therefore we suggest that body weight differences were not an effect of the poly(I:C) treatment, but the effect of the litter size.

On PD 12 pups underwent a battery of juvenile behavioral tests. The first test was the

analysis of USV calls. Although our results show a trend towards a reduced number of USV calls in the HMW pups, no significant differences between groups were observed.

The second performed test was the Negative Geotaxis test, which revealed a significantly longer average time of turning around in females with prenatal LMW poly(I:C) exposure. This is in partial agreement with a similar study by Arsenault et al. (2014), who found that poly(I:C)-induced MIA caused a longer time of turning in poly(I:C) pups, regardless of sex. Arsenault et al. most likely induced stronger MIA as they administered poly(I:C) to dams daily for 3 days. As already mentioned, our results showed that only the female pups in the LMW treatment group were impaired in the Negative Geotaxis test. Deficits in negative geotaxis is indicative of impaired motor development and motivation in young pups and are considered to be indicative of an autism-like phenotype (Ruhela et al., 2019).

The third test performed on PD 12 was the Homing test, which showed a significantly longer return time in the HMW treatment group compared to the control group. This delay can be a result of altered homing reflexes or reduced social behavior of pups after MIA by HMW poly(I:C). Since in this test, significant deficits were not observed in the LMW group, which showed deficits in reflexes in the previous test, we are inclined to relate our results of the Homing test to impaired social behavior. This is the first study testing Homing test in poly(I:C) offspring at the time of writing this thesis. The present results strongly suggest impaired juvenile social behavior after HMW poly(I:C), which can be interpreted as an autism-like phenotype.

Overall, poly(I:C) treatment resulted in altered social behavior in pups, treated by HMW poly(I:C). Reduced homing response and trend towards reduced USV vocalization both point to impairment of HMW pups in interaction with their mother. Autistic behavior too, si first spotted in mother-child interactions (Elder et al., 2002; Meirsschaut et al., 2011). It is possible, that prenatal HMW treatment can model the first signs of autistic behavior: a time when most of the most effective interventions should be first applied.

6.3 Behavior in Adulthood of Poly(I:C) Offspring

Next, this diploma thesis examined the effect of MIA induced by HMW and LMW poly(I:C) administration on GD 14 on the behavior of adult rat offspring. At the age of 3 months, the rats underwent another battery of behavioral tests.

6.3.1 Anxiety Tests

The first adult behavioral test was Elevated Plus Maze. The results of this test revealed increased total time spent in the open arms of the Elevated Plus Maze apparatus in the HMW as well as LMW treatment group compared to the control treatment group. Moreover, the number/frequency of entry into the open arms was also increased in the HMW and LMW groups compared to the control treatment group. These results point to lower anxiety and/or increased exploratory behavior of adult poly(I:C) offspring.

The exploratory behavior together with locomotion activity was also assessed in the Open Field test. In line with EPM results, there was a trend towards higher exploratory behavior in poly(I:C) treated rats. Namely, males in the LMW treatment group spent significantly more time in the center and less time in the corners of the Open Field arena compared to the control treatment group. In addition, the LMW males exhibited greater locomotion activity compared to the control group. This once again points to increased exploratory behavior of poly(I:C) offspring, in this case only in the LMW males. However, although not significant, a tendency of increased exploratory behavior in OF was observed in HMW males as well.

The low anxiety observed in poly(I:C) treated animals in the present work is interesting in the light of high anxiety reported in both autism (reviewed in: White et al., 2009, Vasa et al., 2020) and schizophrenia (reviewed in: Temmingh & Stein, 2015, Karpov et al., 2016). Also, other MIA studies observed high anxiety after prenatal poly(I:C) treatment in both rats (Su et al., 2022) and mice (Babri et al., 2014). To conclude, the observed low anxiety and/or increase in exploratory behavior is difficult to reconcile with the literature that reports high anxiety in autism, schizophrenia and similar MIA studies.

6.3.2 Social Interaction

Present work showed decreased social interaction in poly(I:C) offspring in agreement with previous studies (Malkova et al., 2012; Schwartzer et al., 2013; Talukdar et al., 2020; Su et al., 2022). Specifically, the anogenital sniffing behavior was significantly decreased in males in both the HMW and the LMW groups compared to the control group. The decreased social behavior only in males was previously reported also by Malkova et al. (2012). The decrease in anogenital sniffing was more pronounced in the HMW group than in LMW group. Other aspects of social behavior (non-anogenital sniffing, following, climbing) were not significantly different between the treatment groups. As anogenital interaction requires more effort

compared to non-anogenital, which occurs more incidentally (Donaldson et al., 2018), we suggest less motivation to interact after the prenatal poly(I:C) exposition. In addition, even though not significant, a tendency towards decreased following behavior was observed in the HMW group. In agreement with the study of Becker et al. (2016), the phase of the estrous cycle had no significant effect on the behavior of female rats in any treatment group in the Social Interaction test. Social withdrawal is often observed in patients with schizophrenia (Cullen et al., 2011), driven by reduced drive to engage in social contact (Farina et al., 2022). In autism, social deficits are hallmarks of the disease from its early onset, as already mentioned. Overall, our results indicate that the HMW treatment induces stronger social deficits than LMW treatment, which could be interpreted as autism and schizophrenia phenotypes in adulthood.

6.3.3 Working Memory

The Rewarded Alternation test did not reveal any working memory deficits as the alternation rate did not significantly differ between the treatment groups. Also, there weren't any significant differences in the learning rate between the groups in the training phase. One possible explanation is, that since the error rate in both learning and alternation was quite high in all treatment groups, including controls, the test protocol may not have been optimal. In summary, although there was no difference in working memory, a better-designed test is most likely necessary to assess working memory.

6.3.4 Ketamine-Induced Locomotion

Lastly, the rats underwent the Ketamine-Induced Hyperlocomotion test. Poly(I:C) did not significantly alter the locomotion after ketamine administration in HMW, LMW, or control groups. However, a tendency towards reduced locomotor activity after ketamine administration in poly(I:C) offspring, especially in the HMW treatment group. Unfortunately, due to the limited number of studies assessing ketamine hyperlocomotion after poly(I:C) induced MIA (hyperlocomotion reported by da Silveira et al., 2017) it is very challenging to compare the results. It is necessary to note, that the dose of ketamine used in our experiment was much lower (5 mg/kg) compared to the dose (60 mg/kg) used in Silveira et al. (2017). Also, the locomotion was measured immediately after the treatment in our experiment, whereas Silveira et al. measured locomotion after 15 minutes post-treatment.

6.3.3 Limitations

The project of this diploma thesis has several limitations. First, due to low breeding success, the total number of pregnant dams was smaller than planned. Although the core body temperature measurement showed significantly elevated temperature in the HMW group, the data were obtained from a low number of dams, thus it is not possible to fully confirm this hypothesis. In addition, temperature measurement after poly(I:C) administration may have not been sensitive enough to assess the immune response in individual females, as the treatment by LMW poly(I:C) did not induce noticeable significant temperature elevation compared to the control dams. For this reason, a better choice might have been to measure plasmatic concentrations of pro-inflammatory cytokines (Mueller et al., 2019), despite the risks associated with this method (Kentner et al., 2019). Another potential limitation is the missing test of prepulse inhibition and latent inhibition, which are tests widely used to model behavior relevant to schizophrenia (Weiss & Feldon, 2001; Weiner & Arad, 2009). However, unfortunately, due to the unavailability of the apparatuses in our facility, it was not possible to perform these tests.

In the future, our department will continue to explore the effects of poly(I:C)-induced MIA on offspring. At the same time, our department aims to contribute with a clearer and more detailed description of the different effects of HMW and LMW poly(I:C), including both the difference on the molecular level in addition to effects on the behavior. Our department plans to acquire prepulse and latent inhibition apparatuses and thereby obtain additional valuable data related to models of schizophrenia, which can also be used to test poly(I:C) offspring. Our department will also compare MIA models, such as LPS, with the poly(I:C) MIA model.

Refinement of the MIA model can contribute to research on the early development of psychiatric disorders and clarify the role of environmental factors. This can potentially advance the development of novel therapies and preventive measures in the future.

7 Conclusion

In conclusion, present results showed that HMW poly(I:C) administered on GD 14 induced a stronger immune response in the pregnant dam's body. Next, significant behavioral deficits in the offspring after poly(I:C)-induced MIA both in the early postnatal age and in adulthood were observed. HMW poly(I:C) has been shown to cause social behavior deficits that persisted into adulthood. Because these social deficits emerged early in, these deficits can be associated predominantly with autism phenotypes. Furthermore, it was shown that LMW poly(I:C) causes sex-specific sensorimotor deficits that do not seem to be directly related to autism or schizophrenia.

The results of this diploma thesis show different effects between MIA caused by HMW and LMW poly(I:C) administered on GD 14, thereby confirming that the molecular weight of poly(I:C) is a crucial factor affecting MIA and could strongly contribute to inconsistencies in the results across studies. Overall, the aims of this thesis were achieved.

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