The effect of maternal immune activation and postnatal infection on brain development: a novel approach to schizophrenia research

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Abstract

Schizophrenia is a psychiatric disorder and a major worldwide health problem due to its high societal and economic impact. Despite growing knowledge, the etiology remains unknown. The dual hit hypothesis proposes that a combination of two hits consisting of either genetics or environmental risk factors are required for an individual to develop a psychiatric disorder, such as schizophrenia. The first hit is believed to impair neuronal functioning, increasing the individual's susceptibility to a second hit. Subsequently, the second hit lands more easily, leading to the development of psychiatric disorders such as schizophrenia. Schizophrenia has various risk factors, such as maternal immune activation (MIA) and infection. In addition, recent research also suspects the norepinephrine system (NE) to be linked to schizophrenia. This study therefore aimed to determine if MIA and postnatal infection as a dual hit model resulted in an overactive NE system and affected behavior. This study was performed in female rats to challenge underrepresentation of females in research. MIA was performed by injecting pregnant rats with poly I:C on gestational day 15. Female offspring were injected with LPS during adolescence (postnatal day 36). Clonidine was administered following LPS injection to inhibit the NE system. Behavioral tests were conducted during adolescence and adulthood to assess anhedonia, working memory and social and anxiety-like behavior using the sucrose preference test (SPT), Y maze test (YM), social interaction test (SIT), open field test (OFT) and elevated plus maze test (EPM). MIA + LPS rats traveled a significantly longer distance compared to control rats during adulthood in the EPM test, but not the OFT and YM test. Clonidine rats traveled a significantly shorter distance compared to MIA + LPS rats during adolescence and adulthood in the YM test, but not the OFT and EPM test. MIA rats showed decreased anxiety-like behavior during adulthood in the OFT, as MIA rats spent significantly more time in the 70 cm center of the OFT arena compared to control,LPS and MIA + LPS rats. Moreover, LPS, MIA and MIA + LPS rats showed increased social behavior during adolescence in the SIT, as the interaction time was significantly longer compared to control rats. Furthermore, MIA and LPS did not significantly affect anhedonia-like behavior, working memory, anxiety-like behavior, defecation and exploration.

The present findings observed a possible combined effect of MIA and LPS on increased locomotion, as this effect was not observed in MIA or LPS only rats, nor was it observed in the OFT or YM test. Furthermore, as MIA and LPS solely exerted opposite effects on anxiety-like behavior, a possible dose dependent protective effect of MIA on anxiety-like behavior was also observed. No effect of clonidine on behavior was established, because when there is not a dual hit effect in the first place, significant differences found in clonidine treated groups are not a result from amelioration of behavioral alterations caused by the dual hit. Thus, the dual hit hypothesis of schizophrenia and the NE system need to be further investigated in future studies.

Keywords:
Schizophrenia, dual hit hypothesis, maternal immune activation, lipopolysaccharide, norepinephrine system, clonidine, behavior, rodent model

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Abbreviations

BW	Body weight
CCD	Central Committee on Animal Experiments
CDP	Central Service Laboratory Animals
CLO	Clonidine
CNS	Central nervous system
EPM	Elevated plus maze test
GD	Gestational day
GEE	Generalized estimated equation
IL	Interleukin
IP	Intraperitoneal
IV	Intravenous
IvD	Institutional Animal Care and Use Committee
LC	Locus coeruleus
LPS	Lipopolysaccharide
MIA	Maternal immune activation
NE	Norepinephrine
OFT	Open field test
PET	Positron emission tomography
PND	Postnatal day
Poly I:C	Polyinosinic : polycytidylic acid
PRS	Polygenic risk score
SD	Standard deviation
SIT	Social interaction test
SPT	Sucrose preference test
YM	Y maze test

1 Introduction

Schizophrenia is a psychiatric disorder affecting roughly 1% of the world's population and is considered a major worldwide health problem due to its high societal and economic impact (Janoutová et al., 2016; Mennini et al., 2021; Sher & Kahn, 2019). In addition, schizophrenia causes a higher risk of suicide and reduced life expectancy (Jauhar et al., 2022). Schizophrenia symptoms are classified into positive, negative and cognitive symptoms. Positive symptoms include delusions and hallucinations. Negative symptoms include disrupted emotions like lack of willpower, anhedonia, feeling of emptiness and social isolation. Cognitive symptoms include disorganized behavior-related symptoms, such as memory deficits (Jauhar et al., 2022). Schizophrenia can develop at various ages in both men and women and based on the onset schizophrenia is defined as early/young (onset 13-26 years), middle (26-40 years) or late (>40 years), although it typically develops in early 20s in men and early 30s in women (Matrone et al., 2022; Chen et al., 2018; Li et al., 2016).

The etiology of schizophrenia is still largely unknown, but various risk factors have been identified that can be divided into genetics and environmental risk factors. Genetic risk factors consist of genes involved in neurodevelopmental processes, such as differentiation and synaptic organization, which have been identified through genome-wide association studies (Consortium et al., 2020). Single gene mutation is usually not sufficient, only the combination of genetic mutations, known as a polygenic risk score (PRS), was found to increase the risk of developing schizophrenia (Jauhar et al., 2022). Various environmental risk factors have been associated with schizophrenia, among which maternal immune activation (MIA) and infection (Conway & Brown, 2019)(Table 1). Environmental risk factors can occur either early (prenatally or during childhood) or late (during adolescence and adulthood).

Table 1. Overview of early and late environmental risk factors for the development of schizophrenia, identified in human studies.

Environmental risk factor	Occurrence	Reference
Complications of delivery (C-section[1])	Prenatal	(Cannon et al., 2002)
Pregnancy complications (bleeding, preeclampsia, diabetes)	Prenatal	(Cannon et al., 2002)
Abnormal fetal growth and development	Prenatal	(Cannon et al., 2002)
Compromised prenatal environment (infection, malnutrition, antibiotics, psychosocial stress)	Prenatal	(King et al., 2010; al-Haddad et al., 2019; Cattane et al., 2020)
Winter birth	Postnatal	(Tochigi et al., 2004)
Childhood adversity and trauma (abuse, neglect, bullying)	Childhood	(Varese et al., 2012)
Growing up in urban environment	Childhood	(Vassos et al., 2012)
Sepsis	Juv/Adu[2]	(Daumit et al., 2006; Comim et al., 2015)
Discrimination (job, health system, race)	Juv/Adu[2]	(Zelst, 2008; Varese et al., 2012; Bommersbach et al., 2021)
Early-onset drug abuse	Juv/Adu[2]	(Forti et al., 2014; Marconi et al., 2016)

Immigration	Juv/Adu[2]	(Bourque et al., 2011; Cantor-Graae & Pedersen, 2013)
Socioeconomic factors	Juv/Adu[2]	(Allardyce and Boydell, 2006; Byrne et al., 2004; Paksarian et al., 2015)
Social isolation and social defeat (adversities, trauma)	Juv/Adu[2]	(Stowkowy and Addington, 2012)

[1] = Cesarean section
[2] = Adolescence/Adulthood

Unfortunately, treatment of schizophrenia is not always effective, as current treatment of schizophrenia mostly relies on mitigating positive symptoms through antipsychotic drugs, negative and cognitive symptoms are left unattended (McCutcheon et al., 2020). However, this leaves opportunity for investigation of novel therapies that are also able to mitigate negative and cognitive symptoms so that a broader repertoire of treatment options can be created to touch into the art of personalized medicine. Naturally, the development of a therapeutic that can mitigate positive, negative and cognitive symptoms is also desired. Discovering more about the etiology of schizophrenia and the risk factors involved is crucial in order to progress towards the development of novel (pharmacological) therapies as it will allow to identify novel therapeutic targets (McCutcheon et al., 2020; Jauhar et al., 2022; Patel et al., 2014; Li et al., 2016).

1.1 Dual hit hypothesis

The dual hit hypothesis, also known as the "two hit hypothesis", proposes that a combination of two hits consisting of either genetics or environmental risk factors are required for an individual to develop a psychiatric disorder. The first hit is believed to impair neuronal functioning, increasing the individual's susceptibility to a second hit. Subsequently, the second hit lands more easily, leading to the development of psychiatric disorders, such as schizophrenia (Bayer et al., 1999; Maynard et al., 2001). Human studies observed that individuals with a high PRS (genetic component) that were also exposed to environmental factors, such as drug abuse and childhood adversity, had a higher risk of developing schizophrenia (Guloksuz et al., 2019; Aas et al., 2021). Interestingly, MIA (and inflammation) is increasingly recognized as a key player in the etiology and development of schizophrenia in humans, as synergistic effects between MIA and immuno stressors, such as stressful experiences during pregnancy and childhood trauma, have already been linked to schizophrenia (Han et al., 2021; Conway & Brown, 2019; Meyers, 2019).

Unfortunately, human studies only allow a limited degree of experimental manipulation due to ethical reasons, making etiological research into schizophrenia difficult. Since animal studies do not have this limitation, previous rodent studies have studied a wide selection of risk factors to better understand the underlying mechanisms of schizophrenia, which can be used in future dual hit rodent models (Guerrin et al., 2021). Thanks to rodent studies, the link between MIA and schizophrenia has been strengthened allowing further exploration of the dual hit hypothesis of schizophrenia.

1.2 Role of the immune system

It has been long speculated that inflammation and aberrant functioning of the immune system are involved in the development of schizophrenia (Müller, 2018; Müller et al., 2015). The PRS of schizophrenia includes many genes involved in the immune system, which reflect higher

sensitivity to environmental and genetic risk factors in schizophrenia patients (Werner et al., 2022; Alnæsa et al., 2019). Upregulation of pro-inflammatory cytokines and mediators in the brain, such as interferon, nuclear factor-κB, toll-like receptor and complement signaling, have been found in schizophrenia patients (Müller, 2018; Gayle et al., 2004; van Mierlo et al., 2020). In addition, elevated levels of T-lymphocytes were found in post mortem brain tissue of schizophrenia patients (Sneeboer et al., 2020). Furthermore, *in vivo* positron emission tomography (PET) imaging studies revealed increased activation of microglia, a common neuroinflammation marker, in the hippocampus and gray matter areas of the brains of schizophrenia patients (Doorduin et al., 2009; van Berckel et al., 2008). It has been suggested that MIA in humans, due to stress or infection, can cause neuroinflammation and microglial priming in the fetal brain, altering neurodevelopment which increases vulnerability to an infection for example, ultimately increasing risk of schizophrenia in offspring (Conway & Brown, 2019; Gayle et al., 2004; Malashenkova et al., 2018; Li et al., 2018; Bayer et al., 1999; Maynard et al., 2001; Daumit et al., 2006; Comim et al., 2015). Moreover, neuroinflammation characterized by hyperactive microglia and the complement system can alter neurodevelopmental processes, such as synaptic density, as has been shown in postmortem studies in schizophrenia patients (van Mierlo et al., 2020; Malashenkova et al., 2018; Parellada & Gassó, 2021; Müller, 2018; Sellgren et al., 2019).

1.3 MIA and LPS in dual hit rodent models

Two risk factors used in rodent dual hit models are maternal immune activation (MIA) and injection with lipopolysaccharide (LPS)(Swanepoel et al., 2018). In rodent dual hit models, induction of MIA through injection of polyinosinic-polycytidylic acid (poly(I:C)) or LPS is used as a first hit, whereas postnatal infection through injection of LPS during adolescence is used as a second hit (Möller et al., 2015). Poly(I:C), a synthetic double stranded RNA molecule, and LPS, a glycolipid derived from gram-negative bacteria, are viral and bacterial mimics respectively used to induce inflammation (Haddad et al., 2020; Sandini et al., 2020; Bertani & Ruiz, 2018; Réus et al., 2017).

Previous rodent studies have demonstrated that MIA through poly I:C (gestational day 15) and postnatal infection with LPS (PND3) can cause brain and behavioral abnormalities in adult rats, as MIA caused prepulse inhibition, latent inhibition, social and exploratory deficits and elevated levels of pro-inflammatory cytokines, whereas LPS caused representative symptoms of schizophrenia in humans, namely increased locomotion and decreased social behavior (Sandini et al., 2020; Smith et al., 2007; Gayle et al., 2004; Réus et al., 2017; Möller et al., 2015). MIA through poly I:C as a first hit exacerbated inflammatory brain responses to an immune challenge with LPS injection (PND35) as a second hit in adolescent rats (Clark et al., 2019). However, combining MIA and LPS into a rodent dual hit model can also result in LPS restoring decreased sociability caused by MIA through poly I:C (Reed et al., 2019). Thus interestingly, MIA and LPS can cause similar aberrant functioning of the immune system, as has been shown in schizophrenia patients.

1.4 Role of the norepinephrine system

1.4.1 Norepinephrine and the locus coeruleus

Norepinephrine (NE) is known to exert effects on various types of behavior, such as arousal, attention, vigilance, reward, motivation and also learning and memory (Prokopová, 2010). The majority of NE is produced by the locus coeruleus (LC), a cellular nucleus located in the brainstem. The LC is a key player in the regulation of motivation and cognition, due to its communication with (sub)cortical areas and projections to neuromodulatory systems, such as inhibitory projections to preganglionic parasympathetic neurons and excitatory projections to preganglionic sympathetic neurons (Mäki-Marttunen et al., 2020; Szabadi, 2013). NE binds two distinct types of adrenoceptors, known as alpha- and beta-adrenoreceptors (Prokopová, 2010). Alpha- and beta-adrenoreceptors are located all over the cortex and differ in effects and affinity to NE (Table 2; as shown in Mäki-Marttunen et al., 2020). Moreover, alpha- and beta-adrenoreceptors in the peripherie and the CNS exert opposite effects on blood pressure and cardiac output (Prokopová, 2010; Philipp et al., 2002).

Table 2. Different types of NE receptors and their effects on the cortex (Mäki-Marttunen et al., 2020).

NE receptor	Affinity to NE	Localization	Effect on the cortex
α2-receptors	+++	NE axon terminals (LC[3])	Inhibit NE[5] release
		Dendritic spines of PFC[4] pyramidal neurons	Increase firing of active populations
α1-receptors	++	Dendritic spines of PFC[4] pyramidal neurons	Decrease firing
β1-receptor	+	Dendrites of somatosensory pyramidal neurons	Increase firing
		Hippocampus	Increase firing
NE[5] transporter (NET)		NE[5] axon terminals	Terminate NE[5] and DA[6] transmission

[3] = Locus coeruleus
[4] = Prefrontal cortex
[5] = Norepinephrine
[6] = Dopamine

1.4.2 Norepinephrine and clonidine

Previous studies have shown that aberrant transmission of the norepinephrine (NE) system relates to the etiology of schizophrenia (Borodovitsyna et al., 2017; Fitzgerald, 2014; Yamamoto et al., 2014). Clinical studies further support this, as elevated NE levels were observed in schizophrenia patients (Bondy et al., 1984; Kemali et al., 1982; reviewed in Kammen & Kelley, 1991). Therapeutic studies have demonstrated the benefit of reducing NE levels by using clonidine, an alpha-2 receptor agonist with high affinity for NE that is able to inhibit the LC. Clonidine has been used in the past to successfully treat relapsed and clozapine-resistant schizophrenia patients (Taylor et al., 1988; Lechin et al., 1996; Dardennes et al., 2010). Furthermore, clonidine has also been found to normalize sensori(motor) gating and to improve cognitive and positive symptoms in schizophrenia patients (Oranje & Glenthøj, 2013; Kruiper et al., 2019; Fitzgerald, 2014). It is suggested future studies should investigate whether clonidine can also improve cognitive functioning in schizophrenia.

1.5 Aim of the study

The aim of this study was to investigate if MIA as a first hit can induce vulnerability to an infection (LPS) during adolescence, thus resulting in behavioral alterations related to schizophrenia and if this leads to an overactive NE system. It was hypothesized that MIA as a first hit can induce vulnerability to an infection (LPS) during adolescence, resulting in behavioral alterations related to schizophrenia which in turn can be restored by using clonidine to inhibit the NE system. This study is different from previous studies, as an overactive NE system as a result from the dual hit of schizophrenia has not been researched before. Moreover, this study was conducted in female rats to challenge underrepresentation of females in research, therefore contributing to reducing the knowledge gap between males and females caused by the existing gender bias (Keet et al., 2019).

For the assessment, pregnant rats were exposed to poly I:C to induce maternal immune activation. Female offspring were then injected with either LPS or LPS and clonidine or saline as a control during adolescence. Behavioral alterations related to the positive- (locomotion), negative- (anhedonia, anxiety, social isolation) and cognitive symptoms (working memory) of schizophrenia were assessed with the sucrose preference test (SPT), open field test (OFT), elevated plus maze test (EPM), social interaction test (SIT) and Y maze test (YM).

2 Materials & methods

All experiments and methods that were used in this study are in accordance with the applicable guidelines and regulations. Animal experimentation was conducted according to the European Directive 2010/63/EU and the Law on Animal Experiments of the Netherlands. All experiments were approved by the Dutch Central Committee on Animal Experiments (CCD AVD105002024644) and the Institutional Animal Care and Use Committee of the University of Groningen (IvD 2114644-01-002).

2.1 Animals

Wistar Unilever outbred rats (strain HsdCpb:WU, 11 weeks old) were purchased from Envigo NL (Horst, The Netherlands) and were bred and raised in the Experimental Animal Facility of the University Medical Center Groningen: Central Service Laboratory Animals (CDP). Prior to the breeding, the rats were habituated to the animal facility for at least 7 days. Rats were housed in rooms with controlled temperature (21 ± 2°C) and humidity in a 12-hour light/dark cycle where they had ad libitum access to food and water. Male rats of 3-4 months of age were put with female rats of 3 months of age for 36 hours for breeding. When the males were removed, the female rats were checked for a vaginal plug to ensure the breeding succeeded. Furthermore, pregnancy was determined by measuring weight gain (weekly) and checking physical appearances. Pregnant rats were housed individually under similar housing conditions and were left alone until birth. The female offspring were used for the experiments to challenge underrepresentation of females in research (Keet et al., 2019). The female offspring were weaned, randomly group housed (2 or 3) on postnatal day 21 (PND21) and labeled as experimental or control groups. The rats were considered adults from PND63 onward.

2.2 Experimental design

2.2.1 Study design

Part of the pregnant rats were injected with either saline (control) or poly I:C (MIA) on gestational day (GD) 15. The female offspring was then intraperitoneally (IP) injected with saline or LPS on PND36 to assess the effects of maternal immune activation and postnatal infection on behavior (Figure 1). Clonidine was IP injected on 5 consecutive days, starting on PND36 (day of LPS injection) in a separate group of rats exposed to MIA and LPS. The experimental design was thus made up of 5 different groups: 1 control group (N=8) and 4 experimental groups exposed to LPS (N=9), MIA (N=12), MIA + LPS (N=12) and MIA + LPS + Clonidine (N=9). Short-term (adolescence) and long-term (adulthood) behavioral effects were assessed using the SPT, OFT, EPM test, YM test and SIT to measure anhedonia, locomotion, anxiety, social isolation and working memory respectively. The body weight (BW) was measured a week before LPS injection, on consecutive days after LPS injection and weekly thereafter to monitor animal welfare. Rats that were excluded entirely from the study based on welfare monitoring and litter bias are summarized in Table 3. Data points that failed to be documented are summarized in Table 4. For cohort 7, dual hit and clonidine rats were housed together (rat number 312 + 412 & 410 + 411 + 311). After rat 412 died, rat 312 was housed with rat 511 + 512. Finally, the rats were sacrificed between PND100 and PND104 and brain tissue was collected for post-mortem analysis. Note: this report only includes results from the behavioral experiments during adolescence and adulthood as the study is still ongoing.

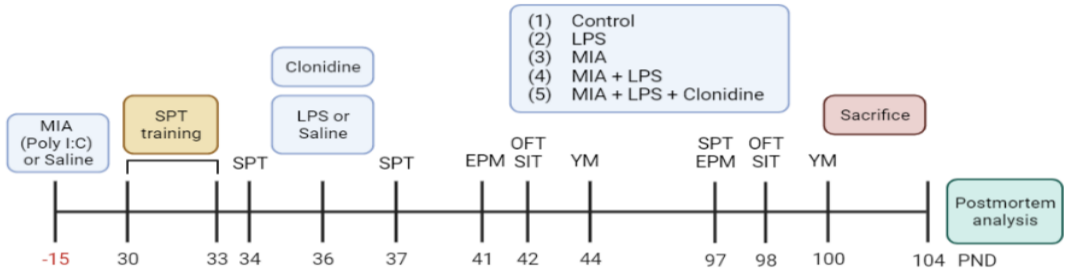


Figure 1. Overview study design of MIA as a first hit and LPS as a second hit. Pregnant rats were injected with Poly I:C to mimic prenatal infection (MIA) as a first hit. On PND36, female offspring were injected with LPS injection as a second hit. Clonidine was administered on 5 consecutive days, starting on PND36. Behavioral tests were performed during adolescence (PND37-44) and adulthood (PND97-100). Poly I:C= polyinosinic : polycytidylic, MIA= maternal immune activation, PND= postnatal day, LPS= lipopolysaccharide, SPT= sucrose preference test, EPM= elevated plus maze test, SIT= social interaction test, OFT= open field test, YM= Y maze test.

Table 3. Overview of rats that were excluded from the study based on welfare monitoring.

Animal ID	Group	Cohort	Reason for exclusion
406	MIA + LPS + CLO	C4	Ate rat 407, got excluded due to possible affected behavior.
407	MIA + LPS + CLO	C4	Died, got eaten by rat 406.
506	MIA	C4	Sacrificed due to litter bias. Animal ID was reused for C6.
507	MIA	C4	Sacrificed due to litter bias. Animal ID was reused for C6.
412	MIA + LPS + CLO	C7	Sacrificed after establishing human end point due to LPS injection.

Table 4. Overview of missing data on body weight.

Date	Cohort	Groups	Animal IDs
06/03	C4	Control, LPS, MIA + LPS, MIA	104, 105, 204, 205, 206, 306, 307, 308, 503, 504, 505
02/04	C6	Control, LPS, MIA + LPS, MIA + LPS + CLO, MIA	106, 107, 108, 207, 208, 209, 309, 310, 408, 409, 506, 507, 508
03/04	C6	Control, LPS, MIA + LPS, MIA + LPS + CLO, MIA	106, 107, 108, 207, 208, 209, 309, 310, 408, 409, 506, 507, 508
09/04	C7	MIA + LPS, MIA + LPS + CLO, MIA	311, 312, 410, 411, 509, 510, 511, 512
10/04	C7	MIA + LPS, MIA + LPS + CLO, MIA	311, 312, 410, 411, 509, 510, 511, 512
22/04	C7	MIA	509, 510

2.2.2 Maternal immune activation (first hit)

On GD15, pregnant rats were anesthetized and intravenously (IV) injected with either 4.0 mg/kg Poly I:C to induce MIA or saline (0.9% NaCl) as a vehicle solution to serve as a control.

2.2.3 Pilot study LPS dosage

Prior to this study, a pilot study was conducted to determine the most effective dosage of LPS. Behavioral effects were assessed with the SPT, EPM test, OFT, SIT and YM test during adolescence after administration of 0.5, 1.0 or 2.0 mg/kg LPS. Due to too little data for statistical analysis, the dosage that caused the biggest differences between treatment groups was deemed the most effective dosage. A dosage of 1.0 mg/kg was concluded to be the most effective.

2.2.4 LPS administration (second hit)

On PND36, rats were IP injected with either 1.0 mg/kg LPS or saline (0.9% NaCl) as a vehicle solution to serve as a control.

2.2.5 Clonidine administration

On PND36-40, rats were IP injected with either 150 µg/kg clonidine or saline (0.9% NaCl) as a vehicle solution to serve as a control.

2.2.6 Sacrifice

Rats were sacrificed between PND 100 and 104. Rats were put in a plastic chamber, in which 5% isoflurane was administered. When deeply anesthetized, rats were put in the table where the nose was connected to a tube with 5% isoflurane to keep them deeply anesthetized. Next, the abdominal wall was cut and kept open using scissors and tweezers, exposing the heart of the rat. The rat was then terminated, performing a heart cut.

2.3 Evaluation of behavior

Behavioral alterations related to positive- (locomotion), negative- (anhedonia, anxiety, social isolation) and cognitive symptoms (working memory) of schizophrenia were assessed with the

sucrose preference test (SPT), open field test (OFT), elevated plus maze test (EPM), Y maze test (YM) and the social interaction test (SIT). For all behavioral experiments except SPT: the rats were habituated to the experimental room for at least 30 minutes before the test was conducted. These experiments were recorded with a camera that was compatible with Ethovision XT 14.0 (Noldus, The Netherlands) and recording software Bandicam. To avoid the presence of any olfactory stimuli, the arenas of these experiments were cleaned with 70% ethanol and dried with paper towels after each trial. To avoid responsive behavior of the rats to auditory stimuli, these experiments were conducted in silence. To prevent bias in data acquisition and habituation of the rats, the order in which the rats were tested for these experiments was randomized.

2.3.1 Sucrose preference test

The SPT was conducted to assess anhedonia. Prior to the experiment, the rats underwent a SPT training on 4 consecutive days, followed by an overnight SPT training in order to get accustomed to the consumption of sucrose and the experimental setup itself. During the training, the rats were subjected to consumption from a weighed bottle with a 1% sucrose solution for one hour each of the four days. During the overnight SPT training and the SPT, the rats were subjected to consumption from two weighed bottles containing either a 1% sucrose solution or water. The morning following the overnight SPT training and the SPT, all bottles were weighed to determine the consumption of both solutions. The bottles were cleaned at the latest when the solutions were 4 days old. The sucrose preference was calculated by the following formula: sucrose preference (%) = sucrose intake / total consumption (sucrose intake + water intake) * 100%. The SPT was conducted on 4 different time points; PND34, PND37, PND41 and PND97. Data from PND34 was retrospectively considered as a training phase due to high variability.

2.3.2 Open field test

The OFT was conducted to assess anxiety-like behavior and locomotion. The open field arena is a round black arena (100 cm diameter) with 4 marked recognition points (North, East, South, West). The rats were placed in the North, facing the wall and were allowed to travel freely for 5 minutes during the experiment. For data analysis, two different center zones were defined: one had a diameter of 70 cm and the other had one of 40 cm. The total distance traveled, time spent in the center and entry frequency of the center were calculated. The time spent in the center was used to measure anxiety-like behavior, whereas the total distance traveled was used to measure locomotion (Kraeuter et al., 2019).

2.3.3 Elevated plus maze test

The EPM test was conducted to assess anxiety-like behavior and locomotion. The EPM test is a plus-shaped maze, consisting of two open arms and two closed arms (50 cm per arm) that are enclosed by high walls. The center (where all arms cross) enables the animal to travel from one arm to another. The plus shaped platform of the EPM test was positioned 62 cm above the ground. The rats were placed in the center, all facing the same closed arm and were allowed to travel freely for 5 minutes during the experiment. Rats that fell off the EPM test were excluded from data analysis (Table 5). For data analysis, the total distance traveled, time spent in the center, open- and closed arms and the entry frequency of the center, open- and closed arms was

calculated. The time spent in the open arms and open arm entries were used to measure anxiety-like behavior, whereas total distance traveled was used to measure locomotion.

Table 5. An overview of rats that were excluded from data analysis of the EPM test.

Animal ID	Group	Cohort	Age	Reason for exclusion
103	Control	C3	Adolescence	Fell off the EPM test during the trial
203	LPS	C3	Adolescence	Fell off the EPM test during the trial
207	LPS	C6	Adolescence	Fell off the EPM test during the trial
308	MIA + LPS	C4	Adolescence	Fell off the EPM test during the trial
312	MIA + LPS	C7	Adolescence	Fell off the EPM test during the trial
411	MIA + LPS + CLO	C7	Adolescence	Fell off the EPM test during the trial
505	MIA	C4	Adolescence	Fell off the EPM test during the trial
305	MIA + LPS	C3	Adulthood	Fell off the EPM test during the trial
306	MIA + LPS	C4	Adulthood	Fell off the EPM test during the trial
308	MIA + LPS	C4	Adulthood	Fell off the EPM test during the trial

2.3.4 Y maze test

The YM test was conducted to assess working memory. The YM test is a Y-shaped maze consisting of 3 arms (50 cm per arm), labeled A, B and C, at an angle of 120°. The rats were placed in the center of the maze facing arm A and were allowed to travel freely for 8 minutes during the experiment. For data analysis, the total distance traveled, total number of entries and the spontaneous alternation index were calculated. Spontaneous alternation was defined as an entry sequence where three different arms are visited consecutively. The spontaneous alternation index was calculated by the following formula: Spontaneous alternation index = (total number of alternations / total number of entries - 2) * 100. No exclusion criteria were used. The spontaneous alternation index was used to assess short-term spatial working memory, as rats generally tend to explore new arms and thus prefer to travel to arms that have not been visited before (Kraeuter et al., 2019). Furthermore, the total distance traveled was used to measure locomotion.

2.3.5 Social interaction test

The SIT was conducted to assess social behavior. Prior to the test, rats were isolated for at least 2 hours. The rats were paired with an unfamiliar partner of roughly the same weight and age and the same gender. Both rats were then placed in opposite corners of the a square arena (50 x 50 cm) and were allowed to freely explore and interact with each other for 5 minutes. The fur of the SIT rats used as partners was marked black so distinction between the rats was easier on video. Different partners were used during adolescence and adulthood to prevent recognition and comfort behavior. The total social interaction time was measured by manually recording the time the experimental animal was interacting with the SIT animal. Social behavior was defined as display of approaching behavior, exploring the partner (grooming, sniffing, licking) and

playing or fighting behavior (pinning, pouncing, fighting). Additionally, the number of plays and fights was scored. The videos of the adolescent control rats were watched once as training for scoring the SIT. For data analysis, 10 blind videos were selected and scored by 3 separate researchers followed by statistical analysis to rule out an observer bias. Likewise, all videos were watched twice to rule out self-inconsistency. Lastly, if two analyses of the same rat differed more than 30 seconds, a third analysis was conducted after which the two most similar trials were used for data analysis. To calculate the interaction time (%), the mean of both trials was used. The trial for adult rat 411 was excluded as the SIT rat was trying to escape the cage by jumping up the walls, thereby not paying attention to adult rat 411 the entire trial.

2.3.6 Excrement scoring

Excrement scoring was used to assess anxiety-like behavior. The number of rat droppings was counted in the EPM test, OFT and YM test. The number of droppings was used as a separate parameter for data analysis, as rats tend to defecate more when experiencing anxiety (Ferré et al., 1995).

2.3.7 Exploration

Exploration was measured to assess refuge seeking. Heatmaps of the OFT were created to show whether the rats spent most time in the spot where they were put in the OFT arena. This spot was then considered as a "safe spot". The safe spot for the OFT was defined as the quarter surrounding the position in which the rat was placed inside the arena. For data analysis, time spent in the safe zone was calculated. Spending time in the safe spot was defined as refuge seeking. This effect was not observed in the EPM test, as rats spent most time in the center of the maze. This effect was observed in the YM test, as rats spent most time in arm A, however, as the YM test is a less appropriate test for spatial orientation, allowing more room for coincidental occurrences, refuge seeking was not assessed in the YM test.

2.3.8 Grooming behavior/barbering

Peculiar grooming behavior was observed and later identified as (self) barbering. During the study, a part of the rats developed bald(ing) spots on various places on the skin upon reaching adulthood. For welfare purposes and grooming behavior (as a sign of hyperactivity for example) being a potential novel method to assess schizophrenia in rodent models, this phenomenon was documented and further identified by creating a grooming risk area profile using pictures identifying areas that became bald in groomed rats. By creating a grooming risk area profile, it will become easier for future studies to identify this phenomenon at an early stage. In addition, animal welfare can be improved as bald skin due to grooming/barbering is more prone to wounds and infections.

2.4 Data analysis of behavioral experiments

Data analysis was performed by using Ethovision XT 14.0 software (Noldus, The Netherlands). The arena settings were defined by using templates provided by Ethovision XT 14.0. Automatic detection settings (dynamic subtraction based on subject versus background color) were used in order to detect the animal. The trial control settings were set to start analysis after a delay of 5 seconds (when the animal is put in the arena) after which it lasted for 5 minutes for the EPM test

and OFT and for 8 minutes for the YM test. The parameters that were measured for each experiment were defined in the analysis profile (Table 6). Additionally, heatmaps were created for the OFT and YM test to learn more about the spatial orientation of the rats during the experiments.

Table 6. Parameters used in the analysis of the EPM test, OFT and YM test results with Ethovision XT 14.0 software.

Behavioral test	Parameters
EPM	Total distance traveled; cumulative time spent/entry frequency in the closed arms, open arms, center; cumulative time spent in the arena
OFT	Total distance traveled; velocity; cumulative time spent/entry frequency in the center zone (70 cm and 40 cm) and border zone; cumulative time spent in the arena; cumulative time spent in safe zone
YM	Total distance moved; number of alternations; maximum number of alternations

2.5 Statistical analysis

Statistical analysis was performed using SPSS 23 software (IBM, United States) for data on body weight and behavior. Due to missing data a two factorial designed generalized estimating equation (GEE) was used for pairwise comparisons to assess the effect of MIA and LPS between all groups and timepoints for the BW and the results of the SPT, OFT, EPM test, YM test and the SIT. For all pairwise comparisons, $p<0.05$ was deemed statistically significant. None of the data were adjusted for multiple comparisons. Data representation via graphs was realized using GraphPad Prism 9.1.2. (GraphPad Software, United States), whereas illustrations were created using Biorender.com. Data are presented per test describing the comparison between groups first, then the comparison between timepoints.

3 Results

3.1 The effect of MIA and LPS on behavior

BW: MIA increased body weight whereas LPS injection reduced body weight

No main effect of MIA ($p=0.089$) or LPS ($p=0.129$) was found on body weight.

MIA rats showed a significantly higher body weight at PND29 (+8,6%, $p=0.027$) and 39 (+12,1%, $p<0.001$) compared to control rats. Following LPS injection on PND36, LPS rats showed a significantly lower body weight at PND37 (-10,7%, $p=0.003$), 38 (-9,7%, $p=0.024$) and 40 (-6,9%, $p=0.041$) compared to control rats. Likewise, MIA + LPS rats showed a significantly lower body weight at PND37 (-6%, $p=0.021$) compared to control rats. MIA + LPS rats showed a significantly higher body weight at PND29 (+9,9%, $p=0.013$) compared to control rats (Figure 2).

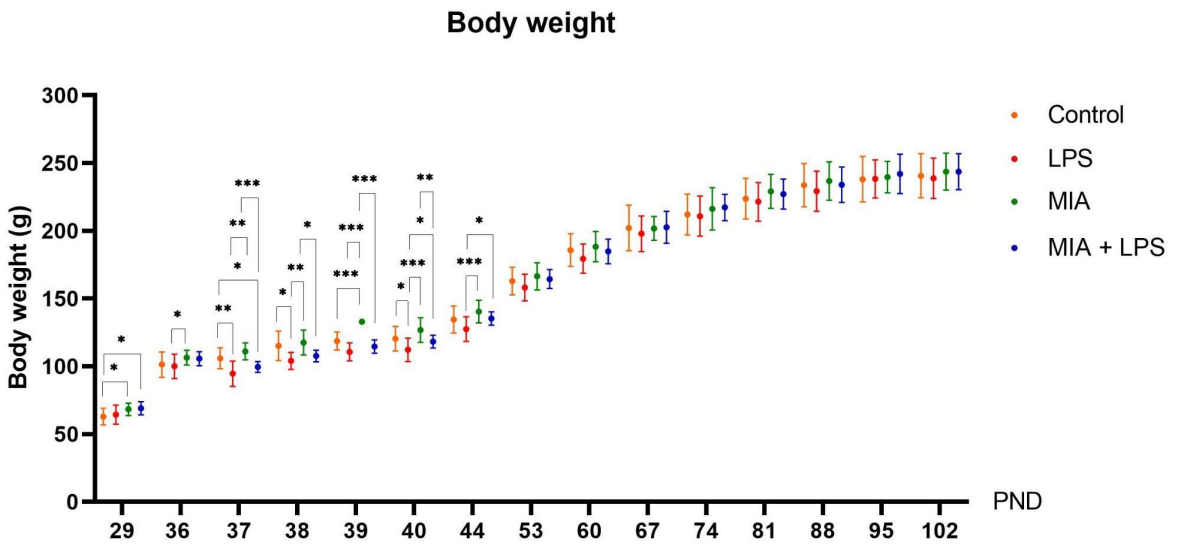


Figure 2. The effect of MIA and LPS on body weight. Comparison between control (N=8), LPS (N=9), MIA (N=12) and MIA + LPS (N=12) groups. Data is presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001.

SPT: MIA and LPS did not significantly modify sucrose preference

No main effect of time (p=0.059), MIA (p=0.785) or LPS (p=0.42) was found on sucrose preference.

No significant differences were observed between MIA + LPS and control rats at any of the timepoints. Following the LPS injection (PND37), LPS and MIA + LPS showed a lower sucrose preference compared to control but not significant (possibly due to spread of control values, whereas LPS (-14,6%, p=0.034) and MIA + LPS rats (-28%, p<0.001) did have a significantly lower sucrose preference compared to MIA rats. No significant differences in sucrose preference were observed between groups on PND41 and 97 (Figure 3A).

OFT: MIA decreased anxiety-like behavior during adulthood, but did not affect locomotion.

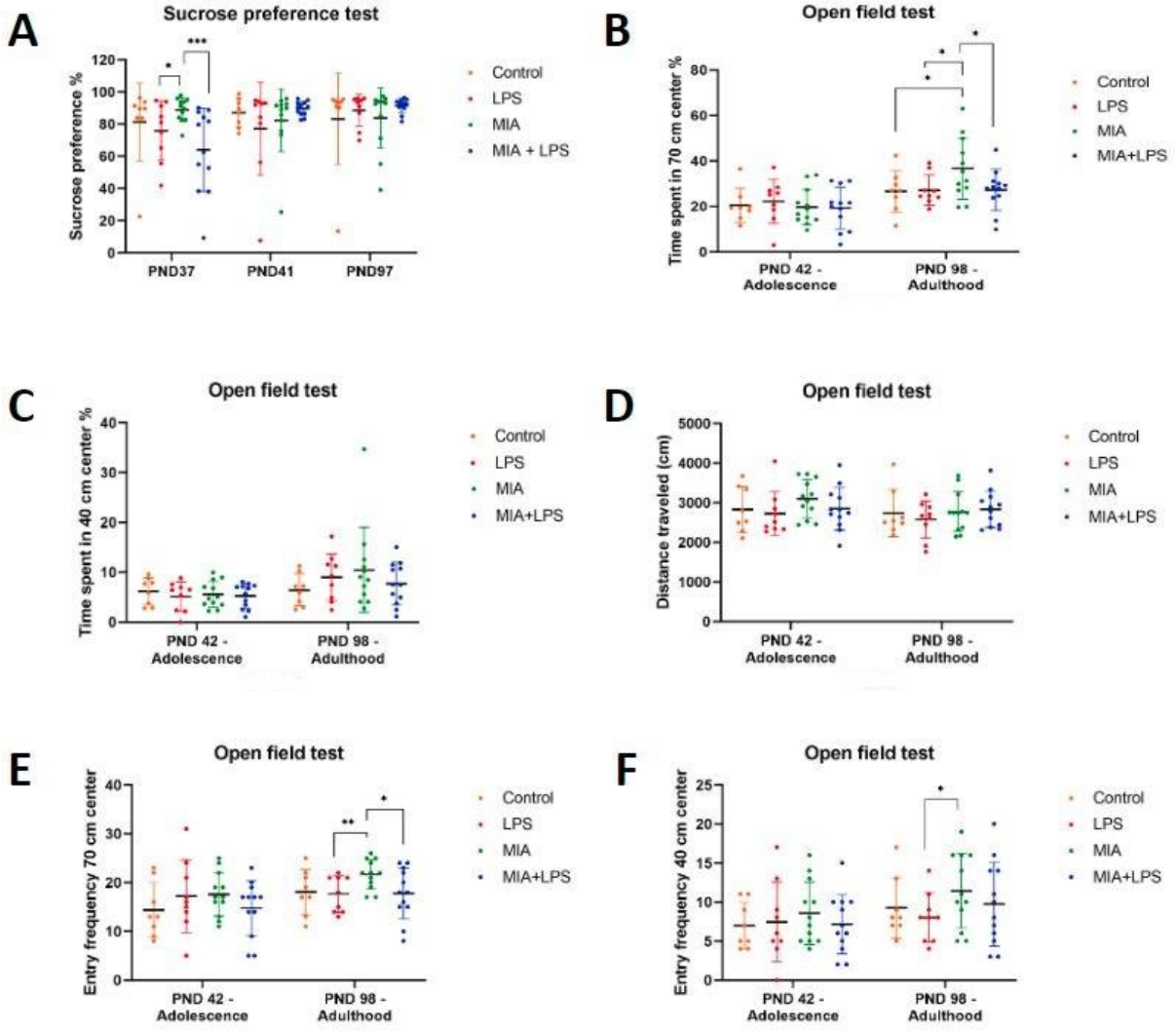
A main effect of time (p<0.001), but not MIA (p=0.435) or LPS (p=0.369) was found on the time spent in the 70 cm center in the OFT. A main effect of time (p=0.004), but not MIA (p=0.48) or LPS (p=0.647) was found on the time spent in the 40 cm center in the OFT. A main effect of time (p=0.003), but not MIA (p=0.385) or LPS (p=0.357) was found on the entry frequency of the 70 cm center of the OFT. A main effect of time (p=0.02), but not MIA (p=0.137) or LPS (p=0.268) was found on the entry frequency of the 40 cm center of the OFT. No main effect of time (p=0.207), MIA (p=0.117) or LPS (p=0.303) was found on the total distance traveled in the OFT. No main effect of time (p=0.66), MIA (p=0.709) or LPS (p=0.611) was found on exploration in the OFT.

During adolescence, no significant differences were observed between groups in any of the parameters assessed in the OFT (Figure 3B-G).

During adulthood, MIA rats spent significantly more time in the 70 cm center compared to control (+37,8%, p=0.036), LPS (+35,2%, p=0.025) and MIA + LPS (+34,5%, p=0.036) rats (Figure 3D). These differences were not observed when a center zone of 40 cm was used (Figure 3C). No significant differences in total distance traveled were observed between groups in the OFT (Figure 3D). Furthermore, MIA rats showed a higher entry frequency in the 70 cm center compared to LPS (+22,6%, p=0.005) and MIA + LPS rats (+22,1%, p=0.019) and a similar

difference compared to control (+18,7%, p=0.052) rats, though not significant (Figure 3E). MIA rats showed a significantly higher entry frequency of the 40 cm center compared to LPS rats (+42,7%, p=0.038), but not to control (+23,4%, p=0.238) or MIA + LPS rats (+17,1%, p=0,402) (Figure 3F). No significant differences in exploration in the OFT were observed between groups (Figure 3G).

When comparing results obtained during adulthood to those obtained during adolescence, only MIA rats (+23,2%, p<0.001) showed a significantly higher entry frequency of the 70 cm center at adulthood. No significant differences in entry frequency were observed in the 40 cm center at adulthood. MIA (+85,7%, p<0.001) and MIA + LPS rats (+40,9%, p=0.004) spent significantly more time in the 70 cm center at adulthood. LPS (+74,6%, p=0.049) and MIA + LPS rats (+46,2%, p=0.038) spent significantly more time in the 40 cm center at adulthood. No significant differences in total distance traveled or exploration in the OFT were observed between timepoints.



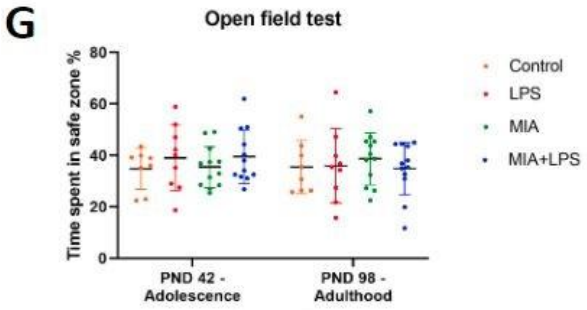


Figure 3. The effect of MIA and LPS on behavior. (A) Sucrose preference (B) Anxiety-like behavior: time spent in the 70 cm center OFT (C) Anxiety-like behavior: time spent in the 40 cm center OFT (D) Locomotion in OFT (E) Anxiety-like behavior: entry frequency of the 70 cm center OFT (F) Anxiety-like behavior: entry frequency of the 40 cm center OFT (G) Exploration in the OFT. Comparison between control (N=8), LPS (N=9), MIA (N=12) and MIA + LPS (N=12). Data is presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001.

EPM test: MIA and LPS increased locomotion during adulthood, but did not affect anxiety-like behavior.

A main effect of time (p=0.041), but not MIA (p=0.191) or LPS (p=0.694) was found on time spent in the closed arms of the EPM test. A main effect of MIA (p<0.001), but not time (p=0.052) or LPS (p=0.209) was found on total distance traveled in the EPM test. No main effect of time (p=0.943), MIA (p=0.514) or LPS (p=0.938) was found on time spent in the open arms of the EPM test. No main effect of time (p=0.098), MIA (p=0.186) or LPS (p=0.223) was found on the entry frequency of the open arms of the EPM test.

During adolescence, no significant differences were observed between groups in any of the parameters assessed in the EPM test (Figure 4A-D).

During adulthood, MIA + LPS rats traveled a significantly longer distance compared to control (+29,2%, p=0.002) and LPS rats (+32,3%, p=0.002)(Figure 4A). No significant differences in the entry frequency of the open arms (Figure 4B) and the time spent in the center (data not shown), open arms (Figure 4C) and closed arms (Figure 4D) were observed between groups.

When comparing results obtained during adulthood to those obtained during adolescence, LPS rats (-36%, p=0.047) spent significantly less time in the open arms at adulthood. LPS rats (-36%, p=0.013) showed a significantly lower entry frequency of entering the open arms at adulthood. LPS rats (-21,2%, p=0.003) traveled a significantly shorter distance at adulthood. LPS (+64,9%, p=0.024), MIA (+22,7%, p=0.037) and MIA + LPS rats (+40,6%, p=0.036) spent significantly more time in the center at adulthood. No significant differences in the time spent in the closed arms were observed between timepoints.

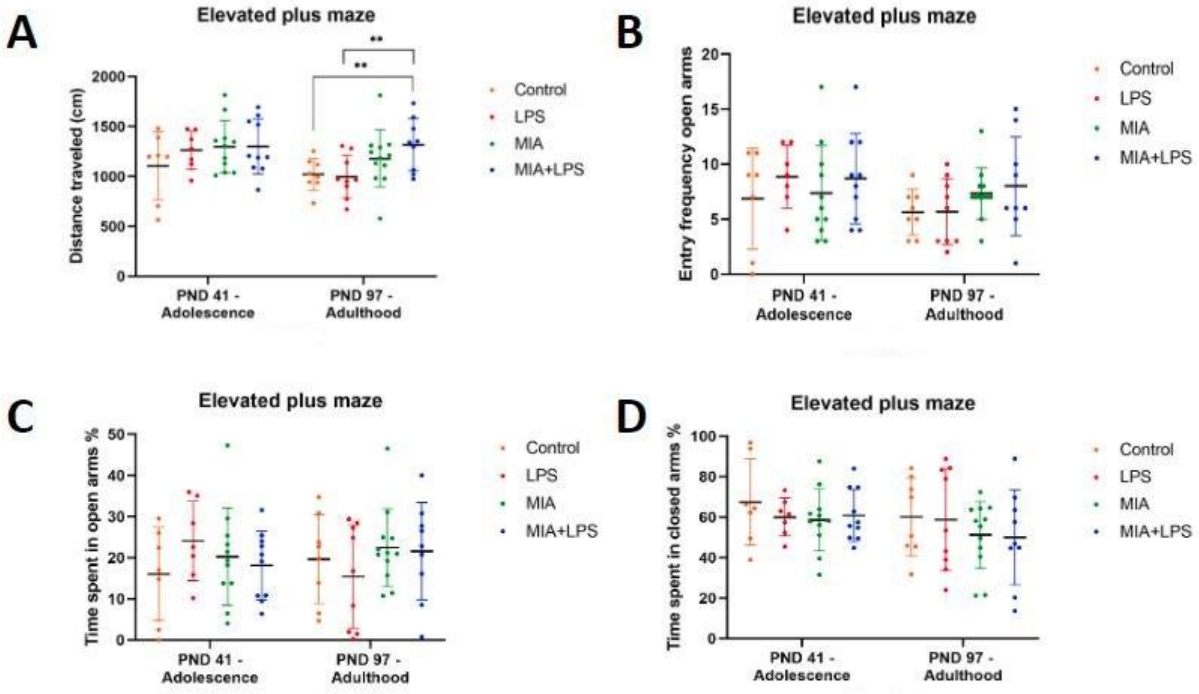


Figure 4. The effect of MIA and LPS on behavior. (A) Locomotion in the EPM test (B) Anxiety-like behavior: entry frequency of the open arms of the EPM test (C) Anxiety-like behavior: time spent in the open arms of the EPM test (D) Anxiety-like behavior: time spent in the closed arms of the EPM test. Comparison between control (N=8), LPS (N=9), MIA (N=12) and MIA + LPS (N=12). Data is presented as mean ± SD. **p<0.01.

YM test: MIA and LPS did not affect working memory.

A main effect of time (p<0.001), but not MIA (p=0.413) or LPS (p=0.154) was found on the total number of entries in the YM test. A main effect of time (p<0.001), but not MIA (p=0.184) or LPS (p=0.473) was found on the total distance traveled in the YM test. No main effect of time (p=0.724), MIA (p=0.878) or LPS (p=0.29) was found on the spontaneous alternation in the YM test.

During adolescence, MIA + LPS rats showed significantly lower number of spontaneous alternation compared to MIA rats (-18%, p=0.047), but not to control rats (-3,6%, p=0.748) or LPS rats -10,6%, p=0,269) (Figure 5A).

During adulthood, MIA + LPS rats traveled a significantly longer distance compared to MIA rats (+13,6%, p=0.030) but not to control (+8,5%, p=0.286) rats (Figure 5B). No significant differences in the total number of entries (Figure 5C) were observed between groups.

When comparing results obtained during adulthood to those obtained during adolescence, no significant differences in spontaneous alternation were observed between timepoints. Control (+28%, p=0.003), LPS (+27,5%, p<0.001), MIA (+15,5%, p=0.001) and MIA + LPS rats (+25,9%, p=0.006) traveled a significantly longer distance at adulthood. Adult LPS rats (+21,7%, p=0.002) showed a significantly higher total number of entries at adulthood.

SIT: MIA, LPS and MIA and LPS increased social behavior during adolescence.

A main effect of time (p>0.001) and LPS (p=0.002), but not MIA (p0.192) was found on social interaction.

During adolescence, LPS (+31,9%, p=0.017), MIA (32,4%, p=0.001) and MIA + LPS rats (+37%, p<0.001) showed a significantly longer interaction time compared to control rats (Figure 5D). These differences were not observed during adulthood.

During adulthood, MIA rats showed a significantly shorter interaction time compared to MIA + LPS rats (-24,7%, p=0.02) (Figure 5D).

When comparing results obtained during adulthood to those obtained during adolescence, LPS (-30,9%, p=0.001), MIA (-46,3%, p<0.001) and MIA + LPS rats (-31,1%, p<0.001) showed a significantly shorter interaction time at adulthood.

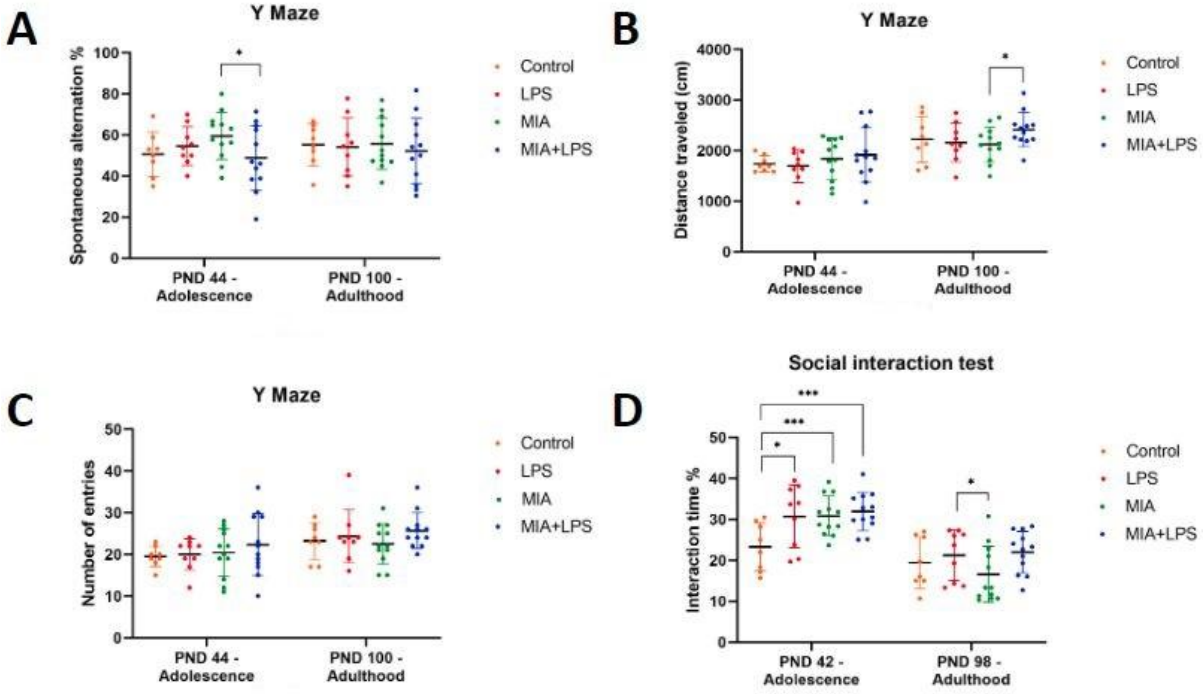


Figure 5. The effect of MIA and LPS on behavior. (A) Working memory: spontaneous alternation in the YM test (B) Locomotion in the YM test (C) Working memory: total number of entries in the YM test (D) Social behavior in the SIT. Comparison between control (N=8), LPS (N=9), MIA (N=12) and MIA + LPS (N=12). Data is presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001.

Excrement scoring: MIA and LPS did not affect defecation.

No main effect of time (p=0.074), MIA (p=0.986) or LPS (p=0.204) was found on defecation in the OFT. No main effect of time (p=0.122), MIA (p=0.867) or LPS (p=0.168) was found on defecation in the EPM test. A main effect of time (p<0.001), but not MIA (p=0.984) or LPS (p=0.274) was found on defecation in the YM test.

During adolescence, MIA rats defecated significantly less compared to MIA + LPS rats (-53,9%, p=0.015), but not control rats (-47,8%, p=0.083) in the OFT (Figure 6A). No significant differences in excrement scoring were observed during adulthood or between timepoints in the OFT (Figure 6A).

No significant differences in excrement scoring were observed between groups in the EPM test (Figure 6B). Adult LPS rats defecated significantly more (+211,2%, p=0.013) than adolescent rats (Figure 6B).

No significant differences in excrement scoring were observed between groups in the YM test (Figure 6C). Adult control (-77,4%, p=0.002), MIA (-66,7%, p<0.001) and MIA + LPS rats (-58,5%, p=0.006) defecated significantly less than adolescent rats (Figure 6C).

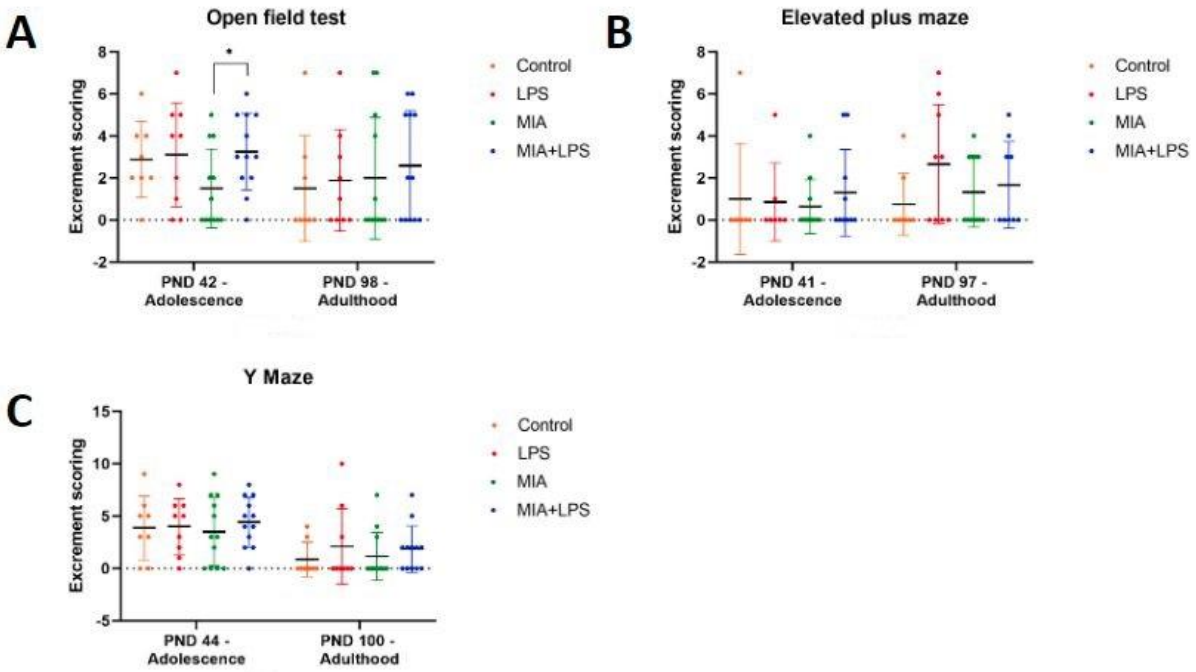


Figure 6. The effect of MIA and LPS on defecation. (A) Excrement scoring in the OFT (B) Excrement scoring in the EPM test (C) Excrement scoring in the YM test. Comparison between control (N=8), LPS (N=9), MIA (N=12) and MIA + LPS (N=12). Data is presented as mean ± SD. *p<0.05.

3.2 The effect of clonidine on behavior of MIA offspring injected with LPS

BW: Clonidine decreased body weight on PND40 and increased body weight on PND60 in rats exposed to MIA and LPS.

No main effect of clonidine (p=0.306) was found on body weight.

Clonidine rats showed a significantly lower body weight on PND40 compared to MIA + LPS rats (-3,6%, p=0.034), whereas the body weight was significantly higher on PND60 (+4,6%, p=0.027)(Figure 7).

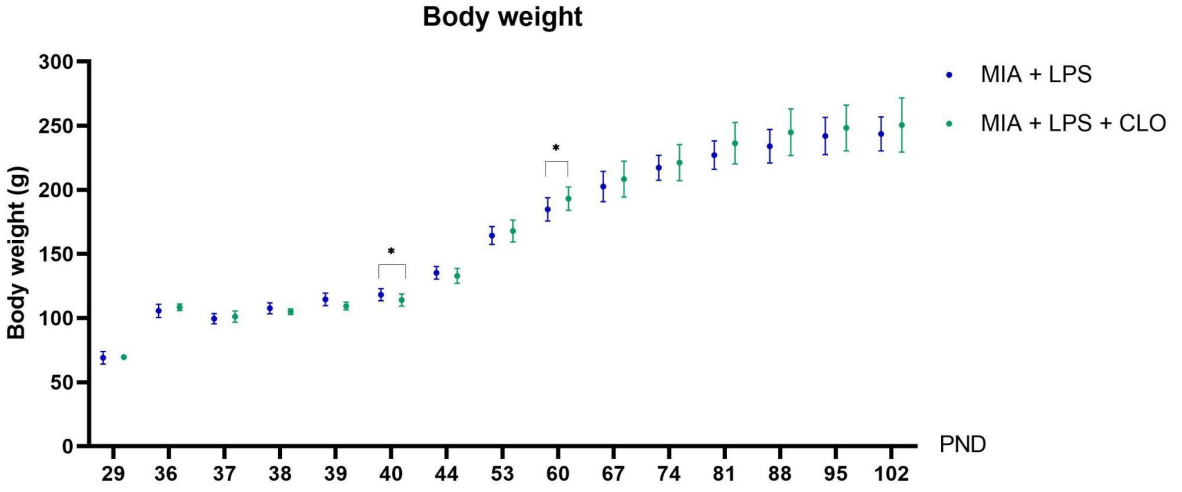


Figure 7. The effect of clonidine on body weight. Comparison between MIA + LPS (N=12) and MIA + LPS + Clonidine (N=9). Data is presented as mean ± SD. *p<0.05.

SPT: Clonidine significantly modified sucrose preference on PND41 in rats exposed to MIA and LPS.

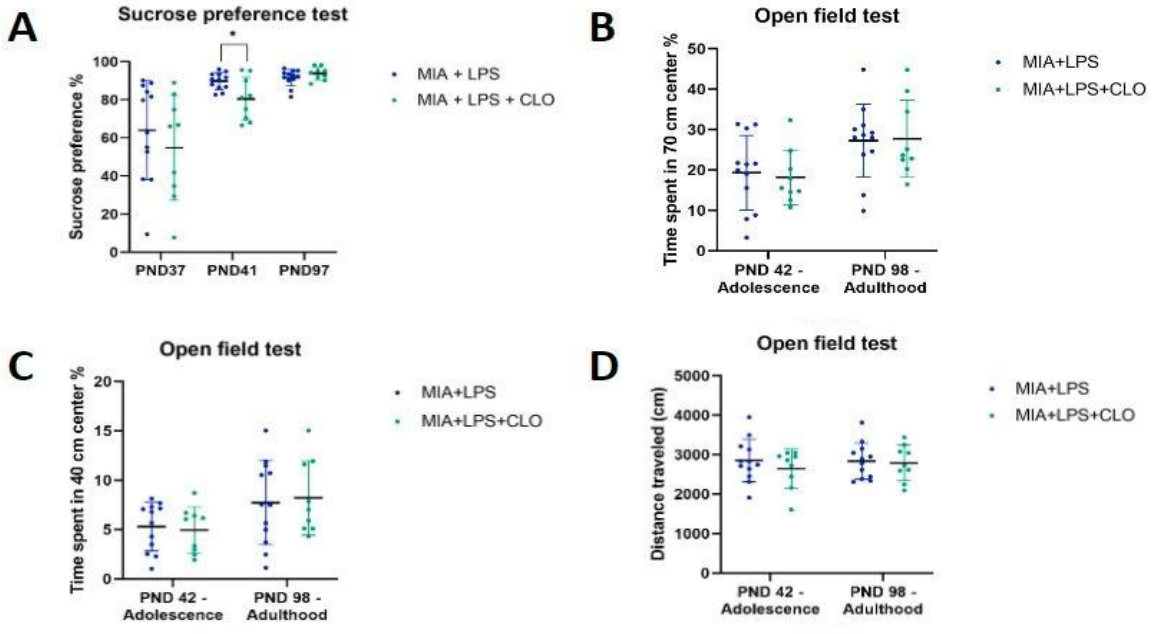
A main effect of time ($p<0.001$), but not clonidine ($p=0.219$) was found on sucrose preference. Clonidine rats showed a significantly lower sucrose preference on PND41 compared to MIA + LPS rats (-10,3%, $p=0.013$)(Figure 8A).

OFT: Clonidine did not affect anxiety-like behavior or locomotion in rats exposed to MIA and LPS.

A main effect of time ($p<0.001$), but not clonidine ($p=0.908$) was found on time spent in the 70 cm center of the OFT. A main effect of time ($p<0.001$), but not clonidine ($p=0.942$) was found on time spent in the 40 cm center of the OFT. A main effect of time ($p<0.001$), but not clonidine ($p=0.766$) was found on the entry frequency of the 70 cm center of the OFT. A main effect of time ($p=0.001$), but not clonidine ($p=0.644$) was found on the entry frequency of the 40 cm center of the OFT. No main effect of time ($p=0.571$) or clonidine ($p=0.445$) was found on the total distance traveled in the OFT. A main effect of clonidine ($p=0.045$), but not time ($p=108$) was found on exploration in the OFT.

No significant differences were observed between groups in any of the parameters in the OFT (Figure 8B-G).

When comparing results obtained during adulthood to those obtained during adolescence, clonidine (+52,6%, $p<0.001$) and MIA + LPS rats (+40,9%, $p=0.004$) spent significantly more time in the 70 cm center at adulthood. Clonidine (+65,3%, $p=0.008$) and MIA + LPS rats (+46,2%, $p=0.038$) also spent significantly more time in the 40 cm center at adulthood. Clonidine rats (+55,1%, $p<0.001$) showed a significantly higher entry frequency of the 70 cm center at adulthood. Adult clonidine rats (+63%, $p=0.001$) also showed a significantly higher entry frequency of the 40 cm center at adulthood. No significant differences in the total distance traveled or exploration in the OFT were observed between timepoints.



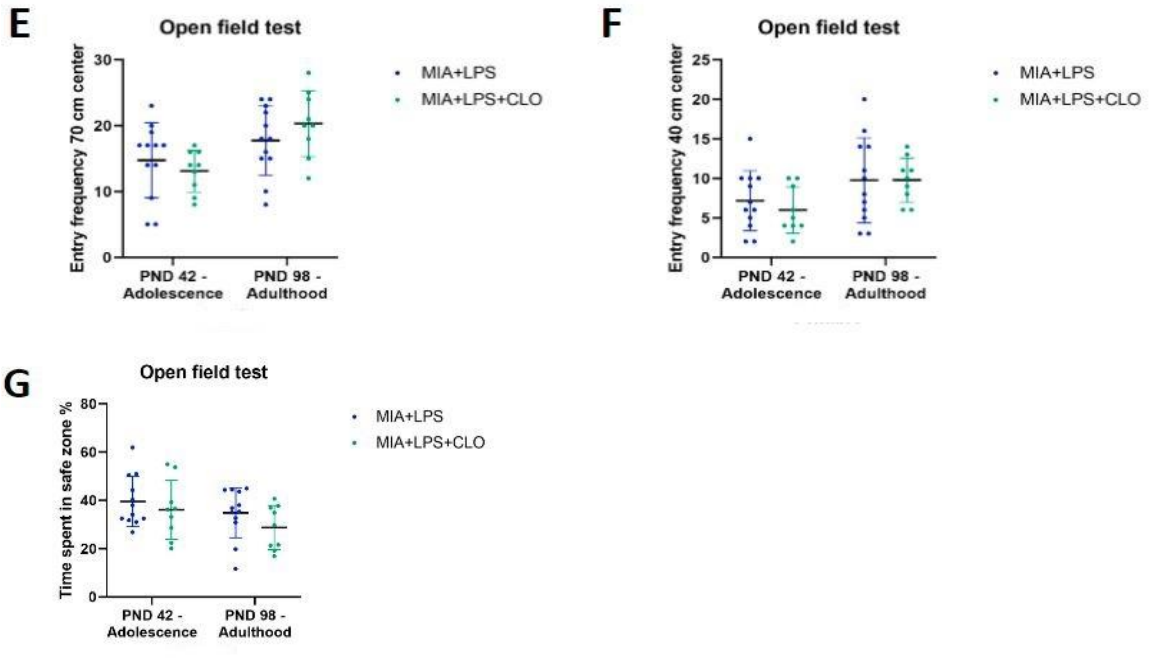


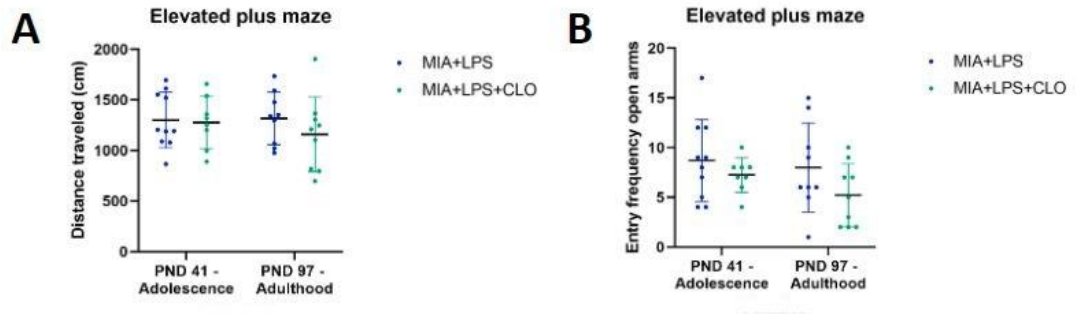
Figure 8. The effect of clonidine on behavior. (A) Sucrose preference (B) Anxiety-like behavior: time spent in the 70 cm center OFT (C) Anxiety-like behavior: time spent in the 40 cm center OFT(D) Locomotion in the OFT (E) Anxiety-like behavior: entry frequency of the 70 cm center OFT (F) Anxiety-like behavior: entry frequency of the 40 cm center OFT (G) Exploration in the OFT. Comparison between MIA + LPS (N=12) and MIA + LPS + Clonidine (N=9). Data is presented as mean ± SD. *p<0.05.

EPM test: Clonidine did not affect anxiety-like behavior or locomotion in rats exposed to MIA and LPS.

No main effect of time (p=0.377) and clonidine (p=0.263) was found on time spent in the open arms of the EPM test. No main effect of time (p=0.837) and clonidine (p=0.157) was found on time spent in the closed arms of the EPM test. No main effect of time (p=0.626) and clonidine (p=0.276) was found on total distance traveled in the EPM test. No main effect of time (p=0.213) and clonidine (p=0.054) was found on entry frequency of the open arms of the EPM test.

No significant differences were observed between groups in any of the parameters in the EPM test (Figure 9A-D).

When comparing results obtained during adulthood to those obtained during adolescence, no significant differences in the entry frequency of the open arms, in the time spent in the open or closed arms, or the total distance traveled were observed. MIA + LPS rats (+40,6%, p=0.036) spent significantly more time in the center at adulthood. This effect was not observed for clonidine rats.



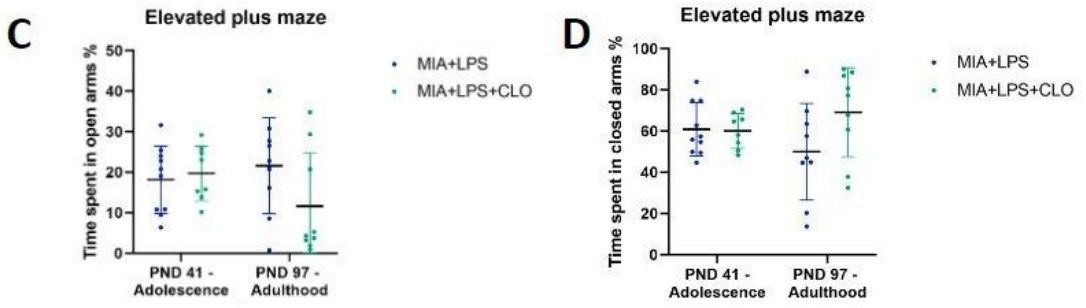


Figure 9. The effect of clonidine on behavior. (A) Locomotion in the EPM test (B) Anxiety-like behavior: entry frequency of the open arms of the EPM test (C) Anxiety-like behavior: time spent in the open arms of the EPM test (D) Anxiety-like behavior: time spent in the closed arms of the EPM test. Comparison between MIA + LPS (N=12) and MIA + LPS + Clonidine (N=9). Data is presented as mean ± SD.

YM test: Clonidine decreased locomotion, but did not affect working memory in rats exposed to MIA and LPS.

A main effect of time (p=0.007), but not clonidine (p=0.503) was found on spontaneous alternation in the YM test. A main effect of time (p=0.005) and clonidine (p=0.028) was found on spontaneous alternation in the YM test. A main effect of time (p<0.001) and clonidine (p=0.003) was found on the total distance traveled in the YM test.

During adolescence (-23%, p=0.033) and adulthood (-13,6%, p=0.02), clonidine rats traveled a significantly shorter distance compared to MIA + LPS rats (Figure 10A). No significant differences in spontaneous alternation were observed between groups (Figure 10B). No significant differences in the total number of entries were observed between groups (Figure 10C).

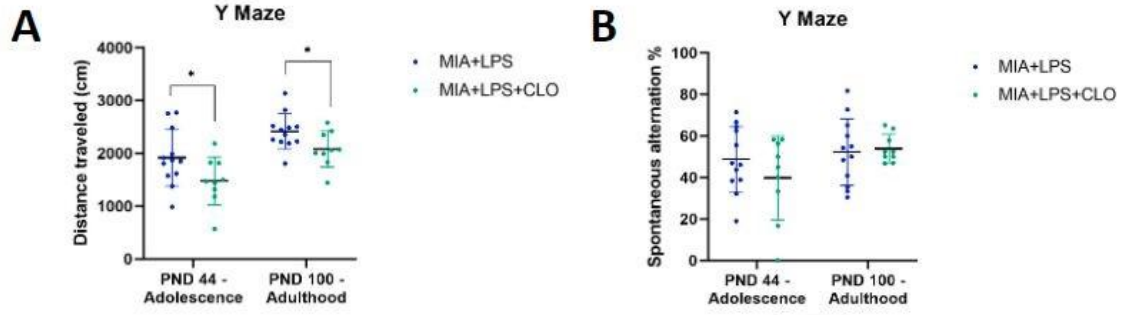
When comparing results obtained during adulthood to those obtained during adolescence, only clonidine rats showed a significantly higher number of spontaneous alternation (+35,6%, p=0.006) at adulthood. Both clonidine (+41,1%, p<0.001) and MIA + LPS rats (+25,9%, p=0.006) traveled a significantly longer distance at adulthood. Clonidine rats showed a higher total number of entries (+29,9%, p=0.002) at adulthood.

SIT: Clonidine did not affect social behavior in rats exposed to MIA and LPS.

A main effect of time (p<0.001), but not clonidine (p=0.329) was found on social interaction.

No significant differences in social interaction were observed between groups (Figure 10D).

When comparing results obtained during adulthood to those obtained during adolescence, both clonidine rats (-33%, p<0.001) and MIA + LPS rats (-31,1%, p<0.001) showed a significantly shorter interaction time at adulthood.



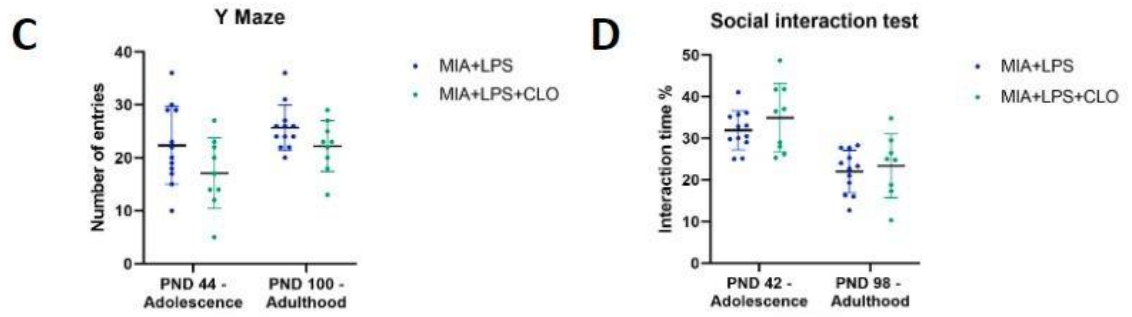


Figure 10. The effect of clonidine on behavior. (A) Locomotion in the YM test (B) Working memory: spontaneous alternation in the YM test (C) Working memory: total number of entries in the YM test(D) Social behavior in the SIT. Comparison between MIA + LPS (N=12) and MIA + LPS + Clonidine (N=9). Data is presented as mean ± SD. *p<0.05.

Excrement scoring: Clonidine did not affect defecation in rats exposed to MIA and LPS.

No main effect of time (p=0.242) or clonidine (p=0.796) was found on defecation in the OFT. No main effect of time (p=0.153) or clonidine (p=0.77) was found on defecation in the EPM test. A main effect of time (p=0.002), but not clonidine (p=0.83) was found on defecation in the YM test. No significant differences in excrement scoring in the OFT were observed between groups and timepoints (Figure 11A).

No significant differences in excrement scoring were observed between groups in the EPM test either (Figure 11B). When comparing adulthood to adolescence, only adult clonidine rats defecated significantly less in the EPM test (-70,4%, p=0.003) (Figure 11B).

No significant differences in excrement scoring were observed between groups in the YM test (Figure 11C). When comparing adulthood to adolescence, only adult MIA + LPS rats (-58,5%, p=0.006) defecated significantly less in the YM test (Figure 11C).

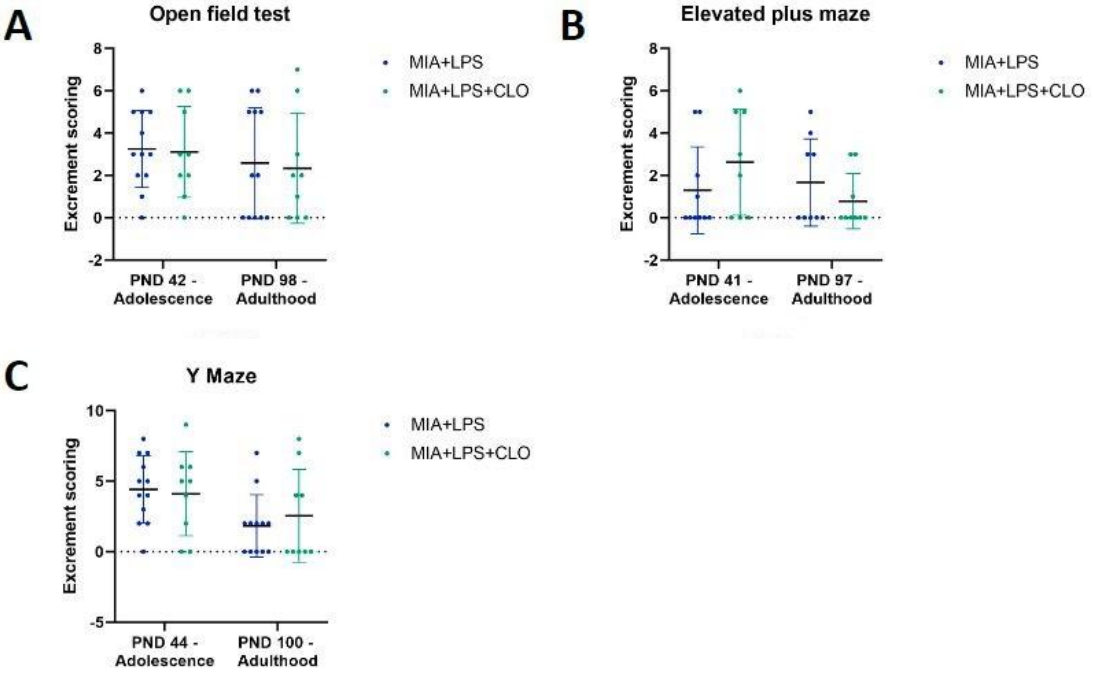


Figure 11. The effect of MIA and LPS on defecation. (A) Excrement scoring in the OFT (B) Excrement scoring in the EPM test (C) Excrement scoring in the YM test. Comparison between MIA + LPS (N=12) and MIA + LPS + Clonidine (N=9). Data is presented as mean ± SD. **p<0.01.

3.3 Identification of grooming behavior

The stomach, shoulders, ears, paws and top of the head were identified as risk areas for excessive grooming.

Typical examples of excessive grooming used for identification are shown in Figure 12.

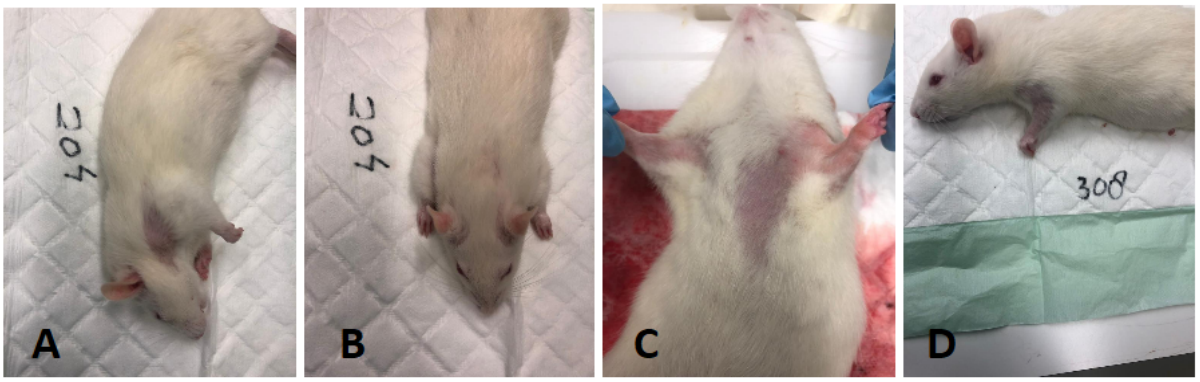


Figure 12. The identification of risk areas of grooming behavior. (A) Identification of the shoulder area by rat 204 (B) Identification of the ear/top of head areas by rat 204 (C) Identification of the stomach area (D) Identification of the paw area.

In an attempt to shine more light on this phenomenon, the rats with balding spots on their skin have been summarized below. Interestingly, 14 out of 15 rats who developed bald spots were treated with MIA, LPS, clonidine or a combination of these. It has been previously documented that oftentimes one rat (hereafter called the 'groomer') grooms/barbers the other rats it's housed with (thus, groomers do not have bald spots on their skin). Potential groomers have been identified in **bold** and groomed/barbered rats are shown in red in Table 7 (Bresnahan et al., 1983). Additionally, self-barbering as a result from health problems or specific diets has also been observed in previous studies, especially in the chest and paw areas (Harkness et al., 2010). It might be interesting for future research to further investigate the behavior of both groomed rats and groomers.

Table 7. Overview of the rats that had bald spots on their skin.

Animal ID	Group	Affected skin area	Potential groomer	Remarks
106	Control	Right paw	106, 107, 108	Nothing on rat 107/108
201	LPS	Both paws	201, **202**, 203	
203	LPS	Left paw	201, **202**, 203	
204	LPS	Both paws, neck and ears	204, 205, **206**	
205	LPS	Both paws and neck	204, 205, **206**	
301	MIA + LPS	Top of head and neck	301, 302, **303**	
302	MIA + LPS	Both ears	301, 302, **303**	
305	MIA + LPS	Both shoulders	**304**, 305	
308	MIA + LPS	Left paw	306, 307, 308	Nothing on rat 306/307

405	MIA + LPS + CLO	Right shoulder, right paw and right side	**403**, 404, 405	Thinning fur on rat 404
502	MIA	Right shoulder	**501**, 502	
506	MIA	Left paw, belly	506, 507, 508	
507	MIA	Both ears	506, 507, 508	
508	MIA	Both ears	506, 507, 508	
509	MIA	Both paws	509, **510**	

4 Discussion

4.1 Overview

In the present study, significant differences in locomotion, anxiety-like behavior and social interaction were observed between groups, whereas no differences in anhedonia-like behavior, working memory, defecation and exploration were observed between groups. Furthermore, significant differences in locomotion, anxiety-like behavior, social interaction and defecation were observed between timepoints, whereas no differences in anhedonia-like behavior, working memory and exploration were observed between timepoints.

4.2 LPS

LPS significantly increased social behavior during adolescence, because LPS rats showed increased social behavior during adolescence in the SIT as the interaction time was significantly longer compared to control. Over time, adult LPS rats showed significantly increased anxiety-like behavior and social interaction and significantly decreased locomotion compared to adolescent LPS rats. LPS rats showed increased anxiety-like behavior over time in the EPM test, as they spent significantly less time in the open arms and showed a significantly lower entry frequency of the open arms whereas no differences over time were observed in the other groups for this parameter. Even though no differences between groups were found for this parameter, it can be seen that contrary to LPS, control rats showed increased time spent in the open arms over time (although not significant), implying that there might be a possible effect of LPS. Furthermore, LPS rats also showed increased defecation, an indicator of anxiety-like behavior, over time as they showed a significantly higher excrement score in the EPM test whereas no differences over time were observed in the other groups for this parameter. In addition, LPS rats decreased locomotion over time in the EPM test, as they traveled a significantly shorter distance whereas no differences over time were observed in other groups for this parameter. LPS rats showed increased social behavior over time in the SIT as the interaction time was significantly longer compared to control.

4.3 MIA

MIA significantly decreased anxiety-like behavior during adulthood and significantly increased social interaction during adolescence. MIA rats showed decreased anxiety-like behavior during adulthood in the OFT, as MIA rats spent significantly more time in the 70 cm center compared to control,LPS and MIA + LPS rats. MIA rats showed increased social behavior during adolescence in the SIT, as the interaction time was significantly longer compared to control rats. Over time, MIA also significantly decreased anxiety-like behavior, but significantly decreased social behavior compared to control. MIA rats showed decreased anxiety-like behavior over time in the OFT, as they showed a significantly higher entry frequency of the 70 cm center whereas no differences over time were observed in other groups for this parameter. MIA rats showed significantly decreased social behavior over time in the SIT, as the interaction time was significantly shorter over time compared to control rats.

A previous rodent study (using MIA through poly I:C as a first hit) revealed increased anxiety-like behavior as they observed male 'two-hit' animals spent significantly less time in the center compared to the control group (no effect in females)(Carlezon et al., 2019). Even though we did not observe a dual hit effect, we observed a decrease in anxiety-like behavior as MIA rats spent significantly more time in the 70 cm center in the OFT compared to control, LPS and MIA + LPS rats. This shows a reduction in anxiety-like behavior in MIA rats, which was abolished compared to the MIA + LPS rats. Besides the difference in parameter, day of LPS administration (PND9 whereas ours was PND36) and gender, this might be due to the dosing of poly I:C and LPS during the present study, as this previous study used 20 mg/kg poly I:C for MIA and 10 mg/kg LPS, whereas we used 4 mg/kg poly I:C for MIA and 1 mg/kg LPS (Carlezon et al., 2019). Thus, a difference in dosing might have caused a different behavioral outcome than hypothesized or found in this previous study (Carlezon et al., 2019). It's possible a lower MIA dose can lead to less anxiety-like behavior and that LPS increases anxiety-like behavior, suggesting a dose dependent protective effect of MIA. Future studies can investigate the effects of different concentrations of poly I:C and/or LPS to answer this and to improve rodent dual hit models similar to ours.

4.4 MIA and LPS

MIA and LPS significantly increased locomotion during adulthood and significantly increased social interaction during adolescence. MIA + LPS rats traveled a significantly longer distance in the EPM test compared to control rats during adulthood, whereas this effect was not observed in the OFT and the YM test. MIA + LPS rats showed increased social behavior during adolescence in the SIT, as the interaction time was significantly longer compared to control rats. Furthermore, no differences in anhedonia-like behavior, working memory, anxiety-like behavior, defecation and exploration were found between MIA + LPS and control rats. Over time, MIA and LPS significantly decreased social interaction, as MIA + LPS rats showed decreased social behavior over time in the SIT as the interaction time was significantly longer over time compared to the control group. No differences in anhedonia-like behavior, working memory, anxiety-like behavior, defecation and exploration were found over time. The present findings suggest a possible combined effect of MIA and LPS on increased locomotion, as this effect was not observed in MIA or LPS only rats, nor was it observed in the OFT or YM test.

Our observation of an increase in locomotion in the EPM test of MIA + LPS compared to control rats during adulthood is in accordance with a previous study as they also observed an increase in locomotion in female 'two-hit' rats during adulthood (Vasconcelos et al., 2021). However, this previous study observed increased locomotion as they measured the number of crossings in the OFT, and used a different dual hit model by using MIA through poly I:C as a first hit and peripubertal stress as a second hit (Vasconcelos et al., 2021). Nevertheless, the results of the present study are further supported by seeing increased locomotion as a sign of hyperactivity as has been shown by previous studies. A previous study with a similar approach, using MIA through LPS as a first hit and LPS injection during adulthood as a second hit, namely observed increased exploration in male MIA offspring (not LPS injected) in the exploratory activity test as a sign of hyperactivity (Chamera et al., 2020). This is further supported by our observation of the grooming/barbering behavior which we hypothesized to be a sign of hyperactivity. Future studies can further investigate this.

Interestingly, we observed increased social behavior during adolescence as LPS, MIA and MIA + LPS rats showed a significantly longer interaction time in the SIT during adolescence. This is not in accordance with previous studies. One rodent study using MIA through poly I:C as a first hit and peripubertal stress as a second hit, observed decreased social interaction in the three chamber paradigm test in male and female 'two-hit' animals (Vasconcelos et al., 2021). Additionally, another rodent study also using MIA through poly I:C as a first hit, but postnatal LPS injection (PND9) as a second hit, also revealed decreased social behavior as they observed a decreased social interaction ratio in male LPS-treated animals in the social approach test (Carlezon et al., 2019). As for the first study, the difference in outcome of social interaction might be explained by the methodical differences of this study compared to ours as this study used a different second hit (peripubertal stress whereas we used LPS injection on PND36), a different dosage of poly I:C to induce MIA (3 doses of 2 mg/kg whereas ours was 1 dosage of 4 mg/kg), a different rodent model (Swiss-Webster mice whereas we used Wistar rats) and a different type of social interaction test to assess social behavior (three chamber paradigm tests whereas we used a social interaction test in a square arena)(Vasconcelos et al., 2021). As for the second study, the difference in outcome of social interaction might also be explained by methodical differences. Besides sex, this study used LPS injection on PND9, 20 mg/kg poly I:C for MIA and 10 mg/kg LPS, C57BL/6 male mice, and a social approach test respectively (Carlezon et al., 2019). The LPS dosage of this study is so high, it can cause a septic shock, resulting in sickness behavior and thus decreased social behavior. Furthermore, both studies used mice and as mice behavior can differ from rat behavior, social behavior can logically differ. All in all, as the LPS doses and the species are the biggest and most relevant differences between these two previous studies and our study, they might have contributed the most to the difference in social behavior.

4.5 Clonidine

Clonidine significantly reduced locomotion during adolescence and adulthood in rats exposed to MIA and LPS and significantly reduced sucrose preference. MIA offspring injected with LPS and clonidine traveled a significantly shorter distance during adolescence and adulthood in the YM test (which was not observed in the dual hit model), whereas this effect was not observed in the OFT and the EPM test. MIA offspring injected with LPS and clonidine showed a significantly reduced sucrose preference on PND41. No differences in working memory, anxiety-like behavior, defecation and exploration were found between MIA and LPS and clonidine rats. No differences

in anhedonia-like behavior, locomotion, working memory, anxiety-like behavior, defecation and exploration were found between MIA and LPS and clonidine rats over time.

Even though the use of clonidine has been shown by previous studies to be an effective method to improve schizophrenia-related symptoms in schizophrenia patients and rodent models, the present study only showed significantly decreased sucrose preference and locomotion in rats exposed to MIA and LPS (Taylor et al., 1988; Lechin et al., 1996; Dardennes et al., 2010). However, if there is not a dual hit effect in the first place, significant differences found in clonidine treated groups are not a result from amelioration of behavioral alterations caused by the dual hit. The significant differences that were observed in clonidine rats are therefore independent of the dual hit. Besides the necessity of introducing a robust dual hit in future studies, for clonidine studies a different alpha-2 receptor agonist should be considered, since alpha-2 receptors come in different subtypes (A, B and C) which achieve different, contrary effects in the body when activated (Philipp et al., 2002). Since clonidine is a non-selective inhibitor, affinity for all 3 subtypes is equal (Cinnamon et al., 2010). Thus, as alpha-2 receptor subtypes A and C occur in the locus coeruleus and exert opposite effects, future studies should assess the differences between stimulation of either of these receptor subtypes (i.e. by usage of prazosin and oxymetazoline) in order to uncover a potential more selective way of ameliorating schizophrenia-related symptoms caused by elevated NE levels (Giovannitti et al., 2015; Bylund & Chacko, 1999).

4.6 Limitations

One limitation of the present study is that the last cohort (C10) including 5 control rats and 3 LPS rats, is not included in the data analysis described in this report. Inclusion of these 8 rats namely improves statistical power. Another limitation is that the rats were not habituated to any of the arenas of the behavioral tests (OFT, EPM test, YM test, SIT), making it possible the rats were not exhibiting schizophrenia-like behavior due to being stressed about the unfamiliarity of the environment (Oummadi et al., 2019). Lastly, this study was conducted only in female rats. Epidemiological studies have observed gender-specific differences in the onset, symptoms and treatment of schizophrenia (Li et al., 2016). Therefore, future studies should consider conducting similar research approaches in males and females simultaneously to avoid gender bias (as done by Carlezon et al., 2019 and Vasconcelos et al., 2021)(Carlezon et al., 2019; Vasconcelos et al., 2021).

5 Conclusion

To conclude, the present findings observed a possible combined effect of MIA and LPS on increased locomotion, as this effect was not observed in MIA or LPS only rats, nor was it observed in the OFT or YM test. Furthermore, as MIA and LPS solely exerted opposite effects on anxiety-like behavior, a possible dose dependent protective effect of MIA on anxiety-like behavior was also observed. No effect of clonidine on behavior was established, because when there is not a dual hit effect in the first place, significant differences found in clonidine treated groups are not a result from amelioration of behavioral alterations caused by the dual hit. Thus, the dual hit hypothesis of schizophrenia and the NE system need to be further investigated in future studies.

6 Bibliography

Aas, M., Alameda, L., di Forti, M., Quattrone, D., Dazzan, P., Trotta, A., Ferraro, L., Rodriguez, V., Vassos, E., Sham, P., Tripoli, G., Cascia, C. la, Barbera, D. la, Tarricone, I., Muratori, R., Berardi, D., Lasalvia, A., Tosato, S., Szöke, A., … Morgan, C. (2021). Synergistic effects of childhood adversity and polygenic risk in first-episode psychosis: the EU-GEI study. *Psychological Medicine*, 1–9. https://doi.org/10.1017/S0033291721003664

al-Haddad, B. J. S., Oler, E., Armistead, B., Elsayed, N. A., Weinberger, D. R., Bernier, R., Burd, I., Kapur, R., Jacobsson, B., Wang, C., Mysorekar, I., Rajagopal, L., & Adams Waldorf, K. M. (2019). The fetal origins of mental illness. *American Journal of Obstetrics & Gynecology*, *221*(6), 549–562. https://doi.org/10.1016/J.AJOG.2019.06.013

Allardyce, J., & Boydell, J. (2006). Environment and Schizophrenia: Review: The Wider Social Environment and Schizophrenia. *Schizophrenia Bulletin*, *32*(4), 592–598. https://doi.org/10.1093/SCHBUL/SBL008

Alnæs, D., Kaufmann, T., van der Meer, D., Córdova-Palomera, A., Rokicki, J., Moberget, T., Bettella, F., Agartz, I., Barch, D. M., Bertolino, A., Brandt, C. L., Cervenka, S., Djurovic, S., Doan, N. T., Eisenacher, S., Fatouros-Bergman, H., Flyckt, L., di Giorgio, A., Haatveit, B., … Westlye, L. T. (2019). Brain Heterogeneity in Schizophrenia and Its Association With Polygenic Risk. *JAMA Psychiatry*, *76*(7), 739–748. https://doi.org/10.1001/JAMAPSYCHIATRY.2019.0257

Bayer, T. A., Falkai, P., & Maier, W. (1999). Genetic and non-genetic vulnerability factors in schizophrenia: the basis of the "Two hit hypothesis." *Journal of Psychiatric Research*, *33*(6), 543–548. https://doi.org/10.1016/S0022-3956(99)00039-4

Bertani, B., & Ruiz, N. (2018). Function and biogenesis of lipopolysaccharides. *EcoSal Plus*, *8*(1). https://doi.org/10.1128/ECOSALPLUS.ESP-0001-2018

Bommersbach, T. J., Rhee, T. G., Stefanovics, E. A., & Rosenheck, R. A. (2021). Comparison of Black and white individuals who report diagnoses of schizophrenia in a national sample of US adults: Discrimination and service use. *Schizophrenia Research*. https://doi.org/10.1016/J.SCHRES.2021.05.017

Bondy, B. ;, Ackenheil, M. ;, Birzle, W. ;, Elbers, R. ;, & Fröhler, M. (1984). *Catecholamines and their receptors in blood: Evidence for alterations in schizophrenia. - PsycNET*. Biological Psychiatry. https://psycnet.apa.org/record/1985-28165-001

Borodovitsyna, O., Flamini, M., & Chandler, D. (2017). Noradrenergic Modulation of Cognition in Health and Disease. *Neural Plasticity*, *2017*. https://doi.org/10.1155/2017/6031478

Bourque, F., van der Ven, E., & Malla, A. (2011). A meta-analysis of the risk for psychotic disorders among first- and second-generation immigrants. *Psychological Medicine*, *41*(5), 897–910. https://doi.org/10.1017/S0033291710001406

Bresnahan, J. F., Kitchell, B. B., & Wildman, M. F. (1983). Facial hair barbering in rats. *Laboratory Animal Science*, *33*(3), 290–291.

Bylund, D. B., & Chacko, D. M. (1999). Characterization of α2 Adrenergic Receptor Subtypes in Human Ocular Tissue Homogenates. *Investigative Ophthalmology & Visual Science*, *40*(10), 2299–2306.

Byrne, M., Agerbo, E., Eaton, W. W., & Mortensen, P. B. (2004). Parental socio-economic status and risk of first admission with schizophrenia. *Social Psychiatry and Psychiatric Epidemiology 2004 39:2*, *39*(2), 87–96. https://doi.org/10.1007/S00127-004-0715-Y

Cannon, M., Jones, P. B., & Murray, R. M. (2002). Obstetric complications and schizophrenia: Historical and meta-analytic review. *American Journal of Psychiatry*, *159*(7), 1080–1092. https://doi.org/10.1176/APPI.AJP.159.7.1080/ASSET/IMAGES/LARGE/K62T2.JPEG

Cantor-Graae, E., & Pedersen, C. B. (2013). Full Spectrum of Psychiatric Disorders Related to Foreign Migration: A Danish Population-Based Cohort Study. *JAMA Psychiatry*, *70*(4), 427–435. https://doi.org/10.1001/JAMAPSYCHIATRY.2013.441

Carlezon, W. A., Kim, W., Missig, G., Finger, B. C., Landino, S. M., Alexander, A. J., Mokler, E. L., Robbins, J. O., Li, Y., Bolshakov, V. Y., McDougle, C. J., & Kim, K. S. (2019). Maternal and early postnatal immune activation produce sex-specific effects on autism-like behaviors and neuroimmune function in mice. *Scientific Reports*, *9*(1). https://doi.org/10.1038/S41598-019-53294-Z

Cattane, N., Richetto, J., & Cattaneo, A. (2020). Prenatal exposure to environmental insults and enhanced risk of developing Schizophrenia and Autism Spectrum Disorder: focus on biological pathways and epigenetic mechanisms. *Neuroscience & Biobehavioral Reviews*, *117*, 253–278. https://doi.org/10.1016/J.NEUBIOREV.2018.07.001

Chamera, K., Szuster-Głuszczak, M., Trojan, E., & Basta-Kaim, A. (2020). Maternal Immune Activation Sensitizes Male Offspring Rats to Lipopolysaccharide-Induced Microglial Deficits Involving the Dysfunction of CD200–CD200R and CX3CL1–CX3CR1 Systems. *Cells*, *9*(7). https://doi.org/10.3390/CELLS9071676

Chen, L., Selvendra, A., Stewart, A., & Castle, D. (2018). Risk factors in early and late onset schizophrenia. *Comprehensive Psychiatry*, *80*, 155–162. https://doi.org/10.1016/J.COMPPSYCH.2017.09.009

Cinnamon Bidwell, L., Dew, R. E., & Kollins, S. H. (2010). Alpha-2 Adrenergic Receptors and Attention—Deficit/Hyperactivity Disorder. *Current Psychiatry Reports*, *12*(5), 366. https://doi.org/10.1007/S11920-010-0136-4

Clark, S. M., Notarangelo, F. M., Li, X., Chen, S., Schwarcz, R., & Tonelli, L. H. (2019). Maternal immune activation in rats blunts brain cytokine and kynurenine pathway responses to a second immune challenge in early adulthood. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *89*, 286. https://doi.org/10.1016/J.PNPBP.2018.09.011

Comim, C. M., Silva, N. C., Patrício, J. J., Palmas, D., Mendonça, B. P., Bittencourt, M. O., Cassol-Jr, O. J., Barichello, T., Zugno, A. I., Quevedo, J., & Dal-Pizzol, F. (2015). Effect of sepsis on behavioral changes on the ketamine-induced animal model of schizophrenia. *Journal of Neuroimmunology*, *281*, 78–82. https://doi.org/10.1016/J.JNEUROIM.2015.02.012

Consortium, T. S. W. G. of the P. G., Ripke, S., Walters, J. T., & O'Donovan, M. C. (2020). Mapping genomic loci prioritises genes and implicates synaptic biology in schizophrenia. *MedRxiv*, 2020.09.12.20192922. https://doi.org/10.1101/2020.09.12.20192922

Conway, F., & Brown, A. S. (2019). Maternal Immune Activation and Related Factors in the Risk of Offspring Psychiatric Disorders. *Frontiers in Psychiatry*, *10*(MAY). https://doi.org/10.3389/FPSYT.2019.00430

Dardennes, R. M., al Anbar, N. N., & Rouillon, F. (2010). Successful augmentation of clozapine-resistant treatment of schizophrenia with clonidine. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*(4), 724–725. https://doi.org/10.1016/J.PNPBP.2010.03.022

Daumit, G. L., Pronovost, P. J., Anthony, C. B., Guallar, E., Steinwachs, D. M., & Ford, D. E. (2006). Adverse events during medical and surgical hospitalizations for persons with schizophrenia. *Archives of General Psychiatry*, *63*(3), 265–272. https://doi.org/10.1001/ARCHPSYC.63.3.267

di Forti, M., Sallis, H., Allegri, F., Trotta, A., Ferraro, L., Stilo, S. A., Marconi, A., la Cascia, C., Marques, T. R., Pariante, C., Dazzan, P., Mondelli, V., Paparelli, A., Kolliakou, A., Prata, D., Gaughran, F., David, A. S., Morgan, C., Stahl, D., … Murray, R. M. (2014). Daily Use, Especially of High-Potency Cannabis, Drives the Earlier Onset of Psychosis in Cannabis Users. *Schizophrenia Bulletin*, *40*(6), 1509–1517. https://doi.org/10.1093/SCHBUL/SBT181

Doorduin, J., de Vries, E. F. J., Willemsen, A. T. M., de Groot, J. C., Dierckx, R. A., & Klein, H. C. (2009). Neuroinflammation in schizophrenia-related psychosis: a PET study. *Journal of Nuclear Medicine : Official Publication, Society of Nuclear Medicine*, *50*(11), 1801–1807. https://doi.org/10.2967/JNUMED.109.066647

Ferré, P., Fernández-Teruel, A., Escorihuela, R. M., Driscoll, P., Corda, M. G., Giorgi, O., & Tobeña, A. (1995). Behavior of the Roman/Verh high- and low-avoidance rat lines in anxiety tests: relationship with defecation and self-grooming. *Physiology & Behavior*, *58*(6), 1209–1213. https://doi.org/10.1016/0031-9384(95)02068-3

Fitzgerald, P. J. (2014). Is elevated norepinephrine an etiological factor in some cases of schizophrenia? *Psychiatry Research*, *215*(3), 497–504. https://doi.org/10.1016/J.PSYCHRES.2014.01.011

Gayle, D. A., Beloosesky, R., Desai, M., Amidi, F., Nuñez, S. E., & Ross, M. G. (2004). Maternal LPS induces cytokines in the amniotic fluid and corticotropin releasing hormone in the fetal rat brain. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *286*(6). https://doi.org/10.1152/AJPREGU.00664.2003

Giovannitti, J. A., Thoms, S. M., & Crawford, J. J. (2015). Alpha-2 Adrenergic Receptor Agonists: A Review of Current Clinical Applications. *Anesthesia Progress*, *62*(1), 31. https://doi.org/10.2344/0003-3006-62.1.31

Guerrin, C. G. J., Doorduin, J., Sommer, I. E., & de Vries, E. F. J. (2021). The dual hit hypothesis of schizophrenia: Evidence from animal models. *Neuroscience & Biobehavioral Reviews*, *131*, 1150–1168. https://doi.org/10.1016/J.NEUBIOREV.2021.10.025

Guloksuz, S., Pries, L.-K., Delespaul, P., Kenis, G., Luykx, J. J., Lin, B. D., Richards, A. L., Akdede, B., Binbay, T., Altınyazar, V., Yalınçetin, B., Gümüş‚-, G., Gümüş‚-, G., O'donovan, M., Rutten, B. P. F., & van Os, J. (n.d.). Manuel Arrojo 28 , Angel Carracedo 29 , Gonzalo López 21,30 , Javier González-Peñas 21,30 , Mara Parellada 21,30 , Nadja P. Maric 16,17 , Cem Atbas‚ogAtbas‚Atbas‚og ˘ lu 31. *Estela Jiménez-López*, *10*.

Haddad, F. L., Patel, S. v., & Schmid, S. (2020). Maternal Immune Activation by Poly I:C as a preclinical Model for Neurodevelopmental Disorders: A focus on Autism and Schizophrenia. *Neuroscience and Biobehavioral Reviews*, *113*, 546–567. https://doi.org/10.1016/J.NEUBIOREV.2020.04.012

Han, V. X., Patel, S., Jones, H. F., Nielsen, T. C., Mohammad, S. S., Hofer, M. J., Gold, W., Brilot, F., Lain, S. J., Nassar, N., & Dale, R. C. (2021). Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: a systematic review. *Translational Psychiatry*, *11*(1). https://doi.org/10.1038/S41398-021-01198-W

Harkness, J. E., Turner, P. v., VandeWoude, S., & Wheler, C. L. (2010). *Biology and Medicine of Rabbits and Rodents* (5th ed.).

Janoutová, J., Janáčková, P., Šerý, O., Zeman, T., Ambroz, P., Kovalová, M., Vařechová, K., Hosák, L., Jiřík, V., & Janout, V. (2016). Epidemiology and risk factors of schizophrenia. *Neuro Endocrinology Letters*, *37*(1), 1–8. https://pubmed.ncbi.nlm.nih.gov/26994378/

Jauhar, S., Johnstone, M., & McKenna, P. J. (2022). Schizophrenia. *The Lancet*, *399*(10323), 473–486. https://doi.org/10.1016/S0140-6736(21)01730-X

Keet, K., Roy, J., & Henry, B. M. (2019). Reporting sex or gender in anatomical research: Which is appropriate? *Clinical Anatomy (New York, N.Y.)*, *32*(5), 697–698. https://doi.org/10.1002/CA.23375

Kemali, D. ;, del Vecchio, M. ;, & Maj, M. (1982). *Increased noradrenaline levels in CSF and plasma of schizophrenic patients. - PsycNET*. Biological Psychiatry. https://psycnet.apa.org/record/1983-01164-001

King, S., St-Hilaire, A., & Heidkamp, D. (2010). Prenatal Factors in Schizophrenia: *Https://Doi.Org/10.1177/0963721410378360*, *19*(4), 209–213. https://doi.org/10.1177/0963721410378360

Kraeuter, A. K., Guest, P. C., & Sarnyai, Z. (2019). The Open Field Test for Measuring Locomotor Activity and Anxiety-Like Behavior. *Methods in Molecular Biology (Clifton, N.J.)*, *1916*, 99–103. https://doi.org/10.1007/978-1-4939-8994-2_9

Kraeuter, A. K., Guest, P. C., & Sarnyai, Z. (2019). The Y-Maze for Assessment of Spatial Working and Reference Memory in Mice. *Methods in Molecular Biology (Clifton, N.J.)*, *1916*, 105–111. https://doi.org/10.1007/978-1-4939-8994-2_10

Kruiper, C., Glenthøj, B. Y., & Oranje, B. (2019). Effects of clonidine on MMN and P3a amplitude in schizophrenia patients on stable medication. *Neuropsychopharmacology 2019 44:6*, *44*(6), 1062–1067. https://doi.org/10.1038/s41386-019-0351-6

LECHIN, F., van der DIJS, B., GOMEZ, F., VALLS, J. M., ACOSTA, E., & AROCHA, L. (1980). Pharmacomanometric studies of colonic motility as a guide to the chemotherapy of schizophrenia. *Journal of Clinical Pharmacology*, *20*(11), 664–671. https://doi.org/10.1002/J.1552-4604.1980.TB01684.X

Li, R., Ma, X., Wang, G., Yang, J., & Wang, C. (2016). Why sex differences in schizophrenia? *Journal of Translational Neuroscience*, *1*(1), 37. /pmc/articles/PMC5688947/

Li, P., Snyder, G. L., & Vanover, K. E. (2016). Dopamine Targeting Drugs for the Treatment of Schizophrenia: Past, Present and Future. *Current Topics in Medicinal Chemistry*, *16*(29), 3385. https://doi.org/10.2174/1568026616666160608084834

Li, J.-W., Zong, Y., Cao, X.-P., Tan, L., & Tan, L. (2018). Microglial priming in Alzheimer's disease. *Annals of Translational Medicine*, *6*(10), 176–176. https://doi.org/10.21037/ATM.2018.04.22

Liu, M. Y., Yin, C. Y., Zhu, L. J., Zhu, X. H., Xu, C., Luo, C. X., Chen, H., Zhu, D. Y., & Zhou, Q. G. (2018). Sucrose preference test for measurement of stress-induced anhedonia in mice. *Nature Protocols 2018 13:7*, *13*(7), 1686–1698. https://doi.org/10.1038/s41596-018-0011-z

Mäki-Marttunen, V., Andreassen, O. A., & Espeseth, T. (2020). The role of norepinephrine in the pathophysiology of schizophrenia. *Neuroscience & Biobehavioral Reviews*, *118*, 298–314. https://doi.org/10.1016/J.NEUBIOREV.2020.07.038

Malashenkova, I. K., Krynskiy, S. A., Ogurtsov, D. P., Mamoshina, M. v., Zakharova, N. v., Ushakov, V. L., Velichkovsky, B. M., & Didkovsky, N. A. (2018). [A role of the immune system in the pathogenesis of schizophrenia]. *Zhurnal Nevrologii i Psikhiatrii Imeni S.S. Korsakova*, *118*(12), 72–80. https://doi.org/10.17116/JNEVRO201811812172

Marconi, A., di Forti, M., Lewis, C. M., Murray, R. M., & Vassos, E. (2016). Meta-analysis of the Association Between the Level of Cannabis Use and Risk of Psychosis. *Schizophrenia Bulletin*, *42*(5), 1262–1269. https://doi.org/10.1093/SCHBUL/SBW003

Matrone, M., Kotzalidis, G. D., Romano, A., Bozzao, A., Cuomo, I., Valente, F., Gabaglio, C., Lombardozzi, G., Trovini, G., Amici, E., Perrini, F., de Persis, S., Iasevoli, F., de Filippis, S., & de Bartolomeis, A. (2022). Treatment-resistant schizophrenia: Addressing white matter

integrity, intracortical glutamate levels, clinical and cognitive profiles between early- and adult-onset patients. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *114*, 110493. https://doi.org/10.1016/J.PNPBP.2021.110493

Maynard, T. M., Sikich, L., Lieberman, J. A., & LaMantia, A. S. (2001). Neural Development, Cell-Cell Signaling, and the "Two-Hit" Hypothesis of Schizophrenia. *Schizophrenia Bulletin*, *27*(3), 457–476. https://doi.org/10.1093/OXFORDJOURNALS.SCHBUL.A006887

McCutcheon, R. A., Reis Marques, T., & Howes, O. D. (2020). Schizophrenia-An Overview. *JAMA Psychiatry*, *77*(2), 201–210. https://doi.org/10.1001/JAMAPSYCHIATRY.2019.3360

Mennini, F. S., Marcellusi, A., Gazzillo, S., Nardone, C., Sciattella, P., Migliorini, R., Trabucco Aurilio, M., Amore, M., & Brugnoli, R. (2021). Economic Impact of Schizophrenia from a Hospital and Social Security System Perspective in Italy. *Clinical Drug Investigation*, *41*(2), 183–191. https://doi.org/10.1007/S40261-020-00991-7

Meyer, U. (2019). Neurodevelopmental Resilience and Susceptibility to Maternal Immune Activation. *Trends in Neurosciences*, *42*(11), 793–806. https://doi.org/10.1016/J.TINS.2019.08.001

Möller, M., Swanepoel, T., & Harvey, B. H. (2015). Neurodevelopmental Animal Models Reveal the Convergent Role of Neurotransmitter Systems, Inflammation, and Oxidative Stress as Biomarkers of Schizophrenia: Implications for Novel Drug Development. *ACS Chemical Neuroscience*, *6*(7), 987–1016. https://doi.org/10.1021/CN5003368

Monji, A., Kato, T. A., Mizoguchi, Y., Horikawa, H., Seki, Y., Kasai, M., Yamauchi, Y., Yamada, S., & Kanba, S. (2013). Neuroinflammation in schizophrenia especially focused on the role of microglia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *42*, 115–121. https://doi.org/10.1016/J.PNPBP.2011.12.002

Müller, N. (2018). Inflammation in Schizophrenia: Pathogenetic Aspects and Therapeutic Considerations. *Schizophrenia Bulletin*, *44*(5), 973–982. https://doi.org/10.1093/SCHBUL/SBY024

Müller, N., Weidinger, E., Leitner, B., & Schwarz, M. J. (2015). The role of inflammation in schizophrenia. *Frontiers in Neuroscience*, *9*. https://doi.org/10.3389/fnins.2015.00372

Oranje, B., & Glenthøj, B. Y. (2013). Clonidine normalizes sensorimotor gating deficits in patients with schizophrenia on stable medication. *Schizophrenia Bulletin*, *39*(3), 684–691. https://doi.org/10.1093/SCHBUL/SBS071

Oummadi, A., Meyer-Dilhet, G., Béry, A., Aubert, A., Barone, P., Mortaud, S., Guillemin, G. J., Menuet, A., & Laugeray, A. (2020). 3Rs-based optimization of mice behavioral testing: The habituation/dishabituation olfactory test. *Journal of Neuroscience Methods*, *332*. https://doi.org/10.1016/J.JNEUMETH.2019.108550

Pacheco-López, G., Giovanoli, S., Langhans, W., & Meyer, U. (2013). Priming of metabolic dysfunctions by prenatal immune activation in mice: relevance to schizophrenia. *Schizophrenia Bulletin*, *39*(2), 319–329. https://doi.org/10.1093/SCHBUL/SBR178

Paksarian, D., Eaton, W. W., Mortensen, P. B., & Pedersen, C. B. (2015). Childhood Residential Mobility, Schizophrenia, and Bipolar Disorder: A Population-based Study in Denmark. *Schizophrenia Bulletin*, *41*(2), 346–354. https://doi.org/10.1093/SCHBUL/SBU074

Parellada, E., & Gassó, P. (2021). Glutamate and microglia activation as a driver of dendritic apoptosis: a core pathophysiological mechanism to understand schizophrenia. *Translational Psychiatry*, *11*(1). https://doi.org/10.1038/S41398-021-01385-9

Patel, K. R., Cherian, J., Gohil, K., & Atkinson, D. (2014). Schizophrenia: Overview and Treatment Options. *Pharmacy and Therapeutics*, *39*(9), 638. /pmc/articles/PMC4159061/

Philipp, M., Brede, M., & Hein, L. (2002). Physiological significance of alpha(2)-adrenergic receptor subtype diversity: one receptor is not enough. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *283*(2). https://doi.org/10.1152/AJPREGU.00123.2002

Prokopová, I. (2010). [Noradrenaline and behavior]. *Ceskoslovenska Fysiologie*, *59*(2), 51–58.

Reed, M. D., Yim, Y. S., Wimmer, R. D., Kim, H., Ryu, C., Welch, G. M., Andina, M., King, H. O., Waisman, A., Halassa, M. M., Huh, J. R., & Choi, G. B. (2020). IL-17a promotes sociability in mouse models for neurodevelopmental disorders. *Nature*, *577*(7789), 249. https://doi.org/10.1038/S41586-019-1843-6

Réus, G. Z., Simões, L. R., Colpo, G. D., Scaini, G., Oses, J. P., Generoso, J. S., Prossin, A. R., Kaddurah-Daouk, R., Quevedo, J., & Barichello, T. (2017). Ketamine potentiates oxidative stress and influences behavior and inflammation in response to lipolysaccharide (LPS) exposure in early life. *Neuroscience*, *353*, 17–25. https://doi.org/10.1016/J.NEUROSCIENCE.2017.04.016

Sandini, T., Greba, Q., Lins, B., & Howland, J. (2020). Maternal Immune Activation with the Viral Mimetic Poly:IC in Pregnant Rats. *Bio-Protocol*, *10*(22). https://doi.org/10.21769/BIOPROTOC.3817

Sellgren, C. M., Gracias, J., Watmuff, B., Biag, J. D., Thanos, J. M., Whittredge, P. B., Fu, T., Worringer, K., Brown, H. E., Wang, J., Kaykas, A., Karmacharya, R., Goold, C. P., Sheridan, S. D., & Perlis, R. H. (2019). Increased synapse elimination by microglia in schizophrenia patient-derived models of synaptic pruning. *Nature Neuroscience*, *22*(3), 374–385. https://doi.org/10.1038/S41593-018-0334-7

Sher, L., & Kahn, R. S. (2019). Suicide in Schizophrenia: An Educational Overview. *Medicina (Kaunas, Lithuania)*, *55*(7). https://doi.org/10.3390/MEDICINA55070361

Smith, S. E. P., Li, J., Garbett, K., Mirnics, K., & Patterson, P. H. (2007). Maternal immune activation alters fetal brain development through interleukin-6. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *27*(40), 10695–10702. https://doi.org/10.1523/JNEUROSCI.2178-07.2007

Sneeboer, M. A. M., van Mierlo, H. C., Stotijn, E., MacIntyre, D. J., Smith, C., Kahn, R. S., Hol, E. M., & de Witte, L. D. (2020). Increased number of T-lymphocytes in post-mortem brain tissue of patients with schizophrenia. *Schizophrenia Research*, *216*, 526–528. https://doi.org/10.1016/J.SCHRES.2019.10.032

Stowkowy, J., & Addington, J. (2012). Maladaptive schemas as a mediator between social defeat and positive symptoms in young people at clinical high risk for psychosis. *Early Intervention in Psychiatry*, *6*(1), 87–90. https://doi.org/10.1111/J.1751-7893.2011.00297.X

Swanepoel, T., Möller, M., & Harvey, B. H. (2018). N-acetyl cysteine reverses bio-behavioural changes induced by prenatal inflammation, adolescent methamphetamine exposure and combined challenges. *Psychopharmacology*, *235*(1), 351–368. https://doi.org/10.1007/S00213-017-4776-5

Szabadi, E. (2013). Functional neuroanatomy of the central noradrenergic system. *Journal of Psychopharmacology (Oxford, England)*, *27*(8), 659–693. https://doi.org/10.1177/0269881113490326

Taylor, J. R., Elsworth, J. D., Garcia, E. J., Grant, S. J., Roth, R. H., & Redmond, D. E. (1988). Clonidine infusions into the locus coeruleus attenuate behavioral and neurochemical changes associated with naloxone-precipitated withdrawal. *Psychopharmacology*, *96*(1), 121–134. https://doi.org/10.1007/BF02431544

Tochigi, M., Okazaki, Y., Kato, N., & Sasaki, T. (2004). What causes seasonality of birth in schizophrenia? *Neuroscience Research*, *48*(1), 1–11. https://doi.org/10.1016/J.NEURES.2003.09.002

van Berckel, B. N., Bossong, M. G., Boellaard, R., Kloet, R., Schuitemaker, A., Caspers, E., Luurtsema, G., Windhorst, A. D., Cahn, W., Lammertsma, A. A., & Kahn, R. S. (2008). Microglia activation in recent-onset schizophrenia: a quantitative (R)-[11C]PK11195 positron emission tomography study. *Biological Psychiatry*, *64*(9), 820–822. https://doi.org/10.1016/J.BIOPSYCH.2008.04.025

van Kammen, D. P., & Kelley, M. (1991). Dopamine and norepinephrine activity in schizophrenia: An intergrative perspective. *Schizophrenia Research*, *4*(2), 173–191. https://doi.org/10.1016/0920-9964(91)90032-M

van Mierlo, H. C., Schot, A., Boks, M. P. M., & de Witte, L. D. (2020). The association between schizophrenia and the immune system: Review of the evidence from unbiased "omic-studies." *Schizophrenia Research*, *217*, 114–123. https://doi.org/10.1016/J.SCHRES.2019.05.028

van Zelst, C. (2008). Which environments for G x E? A user perspective on the roles of trauma and structural discrimination in the onset and course of schizophrenia. *Schizophrenia Bulletin*, *34*(6), 1106–1110. https://doi.org/10.1093/SCHBUL/SBN113

Varese, F., Smeets, F., Drukker, M., Lieverse, R., Lataster, T., Viechtbauer, W., Read, J., van Os, J., & Bentall, R. P. (2012). Childhood Adversities Increase the Risk of Psychosis: A Meta-analysis of Patient-Control, Prospective- and Cross-sectional Cohort Studies. *Schizophrenia Bulletin*, *38*(4), 661–671. https://doi.org/10.1093/SCHBUL/SBS050

Vasconcelos, G. S., dos Santos Júnior, M. A., Monte, A. S., da Silva, F. E. R., Lima, C. N. de C., Moreira Lima Neto, A. B., Medeiros, I. da S., Teixeira, A. L., de Lucena, D. F., Vasconcelos, S. M. M., & Macedo, D. S. (2021). Low-dose candesartan prevents schizophrenia-like behavioral alterations in a neurodevelopmental two-hit model of schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *111*. https://doi.org/10.1016/J.PNPBP.2021.110348

Vassos, E., Pedersen, C. B., Murray, R. M., Collier, D. A., & Lewis, C. M. (2012). Meta-Analysis of the Association of Urbanicity With Schizophrenia. *Schizophrenia Bulletin*, *38*(6), 1118–1123. https://doi.org/10.1093/SCHBUL/SBS096

Werner, M. C. F., Wirgenes, K. V., Shadrin, A. A., Lunding, S. H., Rødevand, L., Hjell, G., Ormerod, M. B. E. G., Haram, M., Agartz, I., Djurovic, S., Melle, I., Aukrust, P., Ueland, T., Andreassen, O. A., & Steen, N. E. (2022). Limited association between infections,

autoimmune disease and genetic risk and immune activation in severe mental disorders. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *116*. https://doi.org/10.1016/J.PNPBP.2022.110511

Yamamoto, K. I., Shinba, T., & Yoshii, M. (2014). Psychiatric symptoms of noradrenergic dysfunction: A pathophysiological view. *Psychiatry and Clinical Neurosciences*, *68*(1), 1–20. https://doi.org/10.1111/PCN.12126