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Published in:
Neurobiology of stress

DOI:
10.1016/j.ynstr.2023.100580

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2023

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Guerrin, C. G. J., Prasad, K., Vazquez-Matias, D. A., Zheng, J., Franquesa-Mullerat, M., Barazzuol, L., Doorduin, J., & de Vries, E. F. J. (2023). Prenatal infection and adolescent social adversity affect microglia, synaptic density, and behavior in male rats. *Neurobiology of stress*, *27*, Article 100580. https://doi.org/10.1016/j.ynstr.2023.100580

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Neurobiology of Stress 27 (2023) 100580

Contents lists available at ScienceDirect

Neurobiology of Stress

journal homepage: www.elsevier.com/locate/ynstr

Prenatal infection and adolescent social adversity affect microglia, synaptic density, and behavior in male rats

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ARTICLE INFO

Keywords:
Maternal immune activation
Social adversity
Microglia
Synaptic density
Synaptophysin
Neurodevelopmental disorders

ABSTRACT

Maternal infection during pregnancy and childhood social trauma have been associated with neurodevelopmental and affective disorders, such as schizophrenia, autism spectrum disorders, bipolar disorder and depression. These disorders are characterized by changes in microglial cells, which play a notable role in synaptic pruning, and synaptic deficits. Here, we investigated the effect of prenatal infection and social adversity during adolescence – either alone or in combination – on behavior, microglia, and synaptic density. Male offspring of pregnant rats injected with poly I:C, mimicking prenatal infection, were exposed to repeated social defeat during adolescence. We found that maternal infection during pregnancy prevented the reduction in social behavior and increase in anxiety induced by social adversity during adolescence. Furthermore, maternal infection and social adversity, alone or in combination, induced hyperlocomotion in adulthood. Longitudinal in vivo imaging with [^{11}C]PBR28 positron emission tomography revealed that prenatal infection alone and social adversity during adolescence alone induced a transient increase in translocator protein TSPO density, an indicator of glial reactivity, whereas their combination induced a long-lasting increase that remained until adulthood. Furthermore, only the combination of prenatal infection and social adversity during adolescence induced an increase in microglial cell density in the frontal cortex. Prenatal infection increased proinflammatory cytokine IL-1β protein levels in hippocampus and social adversity reduced anti-inflammatory cytokine IL-10 protein levels in hippocampus during adulthood. This reduction in IL-10 was prevented if rats were previously exposed to prenatal infection. Adult offspring exposed to prenatal infection or adolescent social adversity had a higher synaptic density in the frontal cortex, but not hippocampus, as evaluated by synaptophysin density. Interestingly, such an increase in synaptic density was not observed in rats exposed to the combination of prenatal infection and social adversity, perhaps due to the long-lasting increase in microglial density, which may lead to an increase in microglial synaptic pruning. These findings suggest that changes in microglia activity and cytokine release induced by prenatal infection and social adversity during adolescence may be related to a reduced synaptic pruning, resulting in a higher synaptic density and behavioral changes in adulthood.

1. Introduction

Exposure to environmental risk factors at different stages of brain development, such as maternal inflammation during pregnancy or childhood social trauma, has been associated with neurodevelopmental psychiatric disorders, such as schizophrenia, autism spectrum disorders (ASD), bipolar disorder and affective psychoses (Brown, 2011; Herbert, 2010; Pedersen and Mortensen, 2001; Tsuchiya et al., 2003). Despite their relatively frequent occurrence, the individual impact of these factors and their effect size in large populations appear relatively weak (Selten et al., 2010; Stilo and Murray, 2010; Varese et al., 2012). To explain how environmental factors with such a relatively modest effect can lead to neurodevelopmental psychiatric disorders, it has been proposed that genetic predisposition or prenatal maternal infection may

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https://doi.org/10.1016/j.ynstr.2023.100580
Received 31 July 2023; Received in revised form 27 September 2023; Accepted 12 October 2023
Available online 19 October 2023
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prime the individual to become more responsive to the pathological effects of a second environmental factor, such as early life social trauma (Bayer et al., 1999; Guerrin et al., 2021; Maynard et al., 2001). Recent epidemiological studies showed that the polygenic risk score and prenatal infection can act in synergy with psychological trauma in peripubertal life to increase the risk of schizophrenia (Aas et al., 2021; Debost et al., 2017; Woolway et al., 2022).

Maternal infection and early-life social trauma can disrupt various neurodevelopmental processes, including neurogenesis, neuronal differentiation, axonal outgrowth, myelination, synaptogenesis, and synaptic pruning (Germann et al., 2021; Howes and McCutcheon, 2017). Altered synaptic pruning has been implicated in neurodevelopmental disorders such as schizophrenia and ASD (Cardozo et al., 2019; Paolicelli et al., 2011). Synaptic pruning, a critical process for the establishment and maturation of functional neural networks, involves the elimination of rarely used synapses while maintaining frequently used connections. This process reaches its peak during adolescence. These alterations in synaptic pruning could be orchestrated by a deviant activity of the immune system characterized by subtle changes in the number and function of microglia and the complement system (Germann et al., 2021; Kim et al., 2021; Koyama and Ikegaya, 2015). The complement system has been reported to trigger the opsonization of synapses, thereby inducing synapse phagocytosis by microglia (Lee et al., 2019). Consequently, abnormal microglial activity and complement system function during critical developmental periods can induce aberrant opsonization of synapses, leading to pathological synaptic pruning that would result in the lower synaptic connectivity observed in schizophrenia (Germann et al., 2021; Koyama and Ikegaya, 2015). Positron emission tomography (PET) imaging and post-mortem studies demonstrated a reduction in synaptic vesicle glycoprotein 2 A (SV2A), synaptophysin, synaptosomal-associated protein (SNAP25) and post synaptic density 95 (PSD-95) protein, all markers of synaptic density, in the frontal cortex and hippocampus of schizophrenic patients (Corradini et al., 2009; Onwordi et al., 2020; Osimo et al., 2019). However, the extent to which maternal infection, social trauma during adolescence, or the combination of these factors may contribute to deviant microglial activity and altered synaptic connectivity in schizophrenia remains unclear. This may be challenging to study prospectively in patients but can be investigated using rodent models.

In this study, we tested whether exposure to maternal infection, social adversity trauma during adolescence and the combination of these factors can affect glial activity and density, synaptic density, and behavior. To achieve maternal infection, we used a well-established rodent model of maternal treatment with the viral mimic polyinosinic:polycytidylic acid (poly I:C) during pregnancy, which is known to capture a wide range of behavioral and neuronal abnormalities in the offspring that are relevant to neurodevelopmental disorders, such as schizophrenia (Brown and Meyer, 2018; Careaga et al., 2017). Social trauma was induced in adolescent rats (PND32-36) through repeated social defeat (RSD), a model known to show behavioral phenotypes relevant to the negative symptoms of schizophrenia (Iñiguez et al., 2014; Vidal et al., 2007; Wang et al., 2021). We assessed symptoms commonly observed in psychopathologies by longitudinally measuring anxiety-like behavior, social behavior, anhedonia, and working memory using the open field (OFT), social interaction (SIT), sucrose preference (SPT), and Y-maze tests, respectively.

To evaluate glial reactivity to these stressors, we performed non-invasive PET imaging with the tracer [^{11}C]PBR28 to measure 18 kDa translocator protein (TSPO) levels. TSPO is a protein found on the outer mitochondrial membrane of glial cells including microglia, and is implicated, among other things, in cholesterol transport and steroid hormone synthesis within mitochondria and opening of the mitochondrial permeability transition pores, which results in energy depletion, release of reactive oxygen species and eventually apoptosis (Biswas et al., 2018; Nutma et al., 2022). TSPO ligands were found to have an anti-inflammatory effect, whereas knock-down of TSPO resulted in exacerbation of inflammation. TSPO levels also serve as a biomarker for microglial density and state as the protein is expressed in microglia and is upregulated when microglia respond to a specific stressor (Beckers et al., 2018; Herrera-Rivero et al., 2015; Van Camp et al., 2021). This upregulation reflects an increase in the number of mitochondria and changes in the mitochondrial membrane that are associated with the increased metabolic demand of microglia when responding to a stimulus. PET imaging enables longitudinal within-group comparisons and non-invasive measurement of TSPO levels in vivo, while also providing translational value as TSPO tracers are clinically used to assess brain inflammation in patients. Potential differences in microglia density as observed with PET were confirmed post-mortem by quantification of immunoreactive cells for the ionized calcium-binding adaptor molecule 1 (Iba1). Additionally, we measured the levels of interleukin 1 beta (IL-1β) and interleukin 10 (IL-10), prototypical pro- and anti-inflammatory cytokines, respectively, in the hippocampus and frontal cortex. Blood samples were collected to assess serum glucocorticoid levels at various time points. Furthermore, we investigated the expression of synaptic proteins—synaptophysin, a major presynaptic protein, and SNAP25, a major postsynaptic protein—in the hippocampus and frontal cortex, two regions strongly implicated in the neuropathology of schizophrenia (Giovanoli et al., 2016a,b).

2. Materials and methods

2.1. Animals

All experiments were performed in accordance with European Directive 2010/63/EU and the Law on Animal experiments in the Netherlands. Wistar rats (strain HsdCpb:WU) were used throughout the study. Only males were included in this study because the RSD model of social stress is well validated in males but not females due to the innate territorial aggression toward other males intruding their territory. A description of the animal housing, breeding and maintenance is provided in the Supplementary Materials.

2.2. Experimental design

Maternal immune activation (MIA) was induced by injecting pregnant rats (n = 6) with the viral mimic polyinosinic:polycytidylic acid potassium salt (poly I:C) on gestational day 15 (GD15). Control rats (n = 6) were injected with saline. Male offspring were subjected to a 5-day repeated social defeat (RSD) or a sham protocol between postnatal day (PND) 32–36.49 rats were randomly divided into four groups: (1) offspring from control mothers (control, n = 13 from 6 litters), (2) offspring from mothers injected with poly I:C (MIA, n = 11 from 6 litters), (3) offspring from control mothers that were exposed to repeated social defeat during adolescence (RSD, n = 13 from 6 litters), and (4) offspring from mothers injected with poly I:C that were exposed to repeated social defeat during adolescence (MIA + RSD, n = 12 from 6 litters). All the male offspring were exposed to all the behavioral experiments and the PET scans (Fig. 1A).

2.3. Maternal immune activation

Poly-I:C (potassium salt: Sigma-Aldrich, Germany) was freshly dissolved in 0.9% NaCl (2 mg/ml) on the day of administration. On GD15, 12 pregnant dams were anesthetized with 5% isoflurane in oxygen and intravenously injected in the tail vein with 4 mg/kg poly-I:C in saline (MIA) or saline (control). Late gestation (GD15) corresponds to the second semester of pregnancy in humans and has been chosen by many previous studies in rats. Once awake, animals were returned to their home cages and checked for possible sickness behavior. A checklist with the methodological details of the MIA model can be found in the supplemental materials (Kentner et al., 2019). To reduce the litter effect, one to three male offspring per litter were used for each group. Animals

were not cross-bred.

2.4. Repeated social defeat

Twelve 20-week old male Long Evans rats (HsdBlu:LE – Envigo, USA) were used as residents and screened for aggressive behavior at least five times before the experiment. Territoriality was encouraged by housing each male resident in a large cage (80*50*40 cm) with an ovariectomized Long Evans female rat for at least one week before the screening. The screening consisted of measuring attack latency and submission time in RSD sessions conducted on five consecutive days to select rats displaying the desired aggressive behavior and exclude rats showing no aggressive behavior (attack latency $>$ 60 s, absence of submission, or residents being submitted), or signs of violence (attack latency $<$10 s and attacking of vital zones).

The RSD protocol was performed between 1 and 4 p.m. One hour before the exposure of the experimental rat to the resident rat, the female Long Evans rat was removed from the resident's cage. The session lasted 1 h and started with the introduction of the experimental rat in the cage of the resident, after which the resident and experimental rats were allowed to interact until the experimental rat showed a submissive posture for at least 5 s or after 10 min of interaction. Then, the experimental rat was placed inside a wire mesh cage and put back in the cage of the resident to allow for visual, auditory, and olfactory interactions for the remainder of the hour. The experimental rat was exposed to five different residents on five consecutive days. Control rats were placed in a wire mesh cage inside a clean cage for a duration of 1 h. The behavior (attack latency and intensity) of the resident rats was similar between control and MIA-exposed intruder animals.

Rats exposed to RSD during adolescence were individually housed during the RSD protocol and until the first PET scan (PND32-42) to prevent the possibility that social interaction during group housing would counteract the effect of social defeat. The groups not exposed to RSD remained group-housed during and after the RSD protocol.

2.5. Behavioral analysis

Various behavioral tests were conducted at different time points to assess anhedonia, social behavior, anxiety, and working memory in rats. The sucrose preference test (SPT) was performed on PND37, 41, and 83 to measure anhedonia. The elevated-plus maze test (EPM) was carried out on PND37 and 83 to measure anxiety and locomotion. The open field test (OFT) was performed on PND40 and 85 to measure anxiety-like behavior and locomotion. The social interaction test was conducted on PND40 and 85 to assess social behavior. The Y-maze test was conducted on PND41 and 86 to assess working memory and locomotion. A detailed description of the behavioral test apparatuses, procedures and analyses is provided in the Supplementary Materials.

2.6. Positron emission tomography

PET was used to measure TSPO levels as a marker of microglia reactivity during adolescence (PND42) and adulthood (PND90). A detailed description of the methods is provided in the Supplementary Materials. In short, rats were anesthetized with isoflurane in oxygen (5% for induction and 2% for maintenance) before intravenous injection of 31.7 $\pm$ 5.3 MBq [^{11}C]PBR28 in a tail vein. After injection, the rats were immediately placed back in their home cage. About 30 min after tracer injection, rats were again anesthetized with isoflurane and positioned into the PET camera (microPET Focus 220, Siemens) for a transmission scan of 10 min followed by an emission scan of 30 min, starting at 45 min after tracer injection.

2.7. Brain collection, and immunohistochemistry

Within 10 min after the last PET scan, rats were perfused with PBS under deep anesthesia, and their brains were collected. The left hemisphere was dissected to isolate the frontal cortex and hippocampus before being snap-frozen in liquid nitrogen. The right hemisphere was fixed for 48 h in 4% PFA at room temperature before being dehydrated in 25% sucrose solution at 4 °C, embedded with optimal cutting temperature OCT compound and stored at −80 °C.

To ascertain the effects of MIA, RSD and their combination on microglia density, we performed immunohistochemical analysis of Iba1, a cellular marker expressed in the entire microglia population (Ransohoff and Perry, 2009). Microglia densities and distance of the microglia to their nearest neighbor in the parietal and frontal cortices were determined by counting all Iba1 positive cells in 6–10 regions of interest (ROI) per cortical region per animal. Four to six rats per group were randomly selected for analysis. A detailed description of the immunohistochemistry and microglial density and spatial distribution analysis can be found in the Supplementary materials.

2.8. ELISA for plasma corticosterone and for brain synaptophysin, SNAP25, IL-1β, and IL-10

The description of the ELISA for plasma corticosterone is provided in the Supplementary materials. Blood samples were collected from the tail vein, prior to each injection of the PET tracer. Blood samples were immediately centrifuged at 5000 g for 3 min. The supernatant (plasma) was collected, frozen in liquid nitrogen and stored at −80 °C. Plasma corticosterone measurement was performed using an enzyme-linked immunoassay (ELISA) using a commercially available kit (Arbor Assays, DetectX Corticosterone Immunoassay kit) according to the manufacturer's recommendations.

Snap-frozen samples of the hippocampus and frontal cortex were homogenized with a tissue homogenizer, the cell suspension was sonicated, the homogenate was centrifuged at 10,000×*g* for 5 min and the supernatant was collected. Synaptophysin, SNAP25, IL-1β, and IL-10 protein levels were measured using commercially available kits (Abbexa Rat synaptophysin, SNAP25, IL-1β, and IL-10 ELISA kit) according to the manufacturer's recommendations.

2.9. Statistical analysis

Statistical analyses of body weight, behavior, and PET data were performed using SPSS (IBM SPSS Statistics, Version 22.0). A generalized estimating equation (GEE) analysis was performed, using 'MIA', 'RSD' and 'time' as factors for longitudinal statistical analyses as this analysis can account for missing data and was adjusted for multiple comparisons using the "least significant difference post-hoc correction". Wald Chi-square (W) and degrees of freedom (df) are presented for GEE analysis. A one-way ANOVA, followed by a Tukey's multiple comparisons post hoc test whenever appropriate, was performed using GraphPad 8 software to assess differences in Iba1 staining, inflammatory cytokines, and synaptic protein levels between groups, as these parameters were assessed at only a single time point and no data was missing. F value and degree of freedom are presented for one-way ANOVA. An unpaired *t*-test was performed to assess differences in RSD severity (attack and submission latency). Direct statistical comparison between MIA and RSD are not shown. The data are presented as mean $\pm$ standard deviation (SD). In the figures, white empty circles represent control rats, yellow (MIA) and blue (RSD) half-filled circles represent rats exposed to a single stressor, while the green (MIA + RSD) filled circles represent rats exposed to the combination of the two stressors.

3. Results

3.1. Social defeat protocol

Average attack latency (RSD = 76 $\pm$ 68 s, MIA + RSD = 84 $\pm$ 79 s, p = 0.57) was not significantly different between RSD and MIA + RSD rats

(Supplementary Fig. S1).

3.2. MIA increased body weight during adolescence

The body weight of all groups increased over time (W = 16,817, df = 10, p < 0.001, Fig. 1B). We observed no main effect of MIA and RSD on body weight. Pairwise analysis (W = 6,037,350, df = 43, p < 0.001) revealed that MIA rats had a higher body weight than control (+9%, p = 0.037) and MIA + RSD rats (+8%, p = 0.011) on PND36. A higher body weight of MIA rats compared to control (+7%, p = 0.002), and MIA + RSD rats (+5%, p = 0.050) was also observed on PND49.

3.3. No effect of MIA or RSD on sucrose preference

The sucrose preference test was used to assess anhedonia (Fig. 1C). We observed a RSD*Time interaction (W = 7.4, df = 2, p = 0.025) in sucrose preference. We observed no significant differences in sucrose preference between the experimental groups and control rats at any time point. On PND37, MIA + RSD rats (−10%, W = 40.9, df = 11, p = 0.010) had lower sucrose consumption than MIA rats. This difference was not observed anymore on PND41 and PND83.

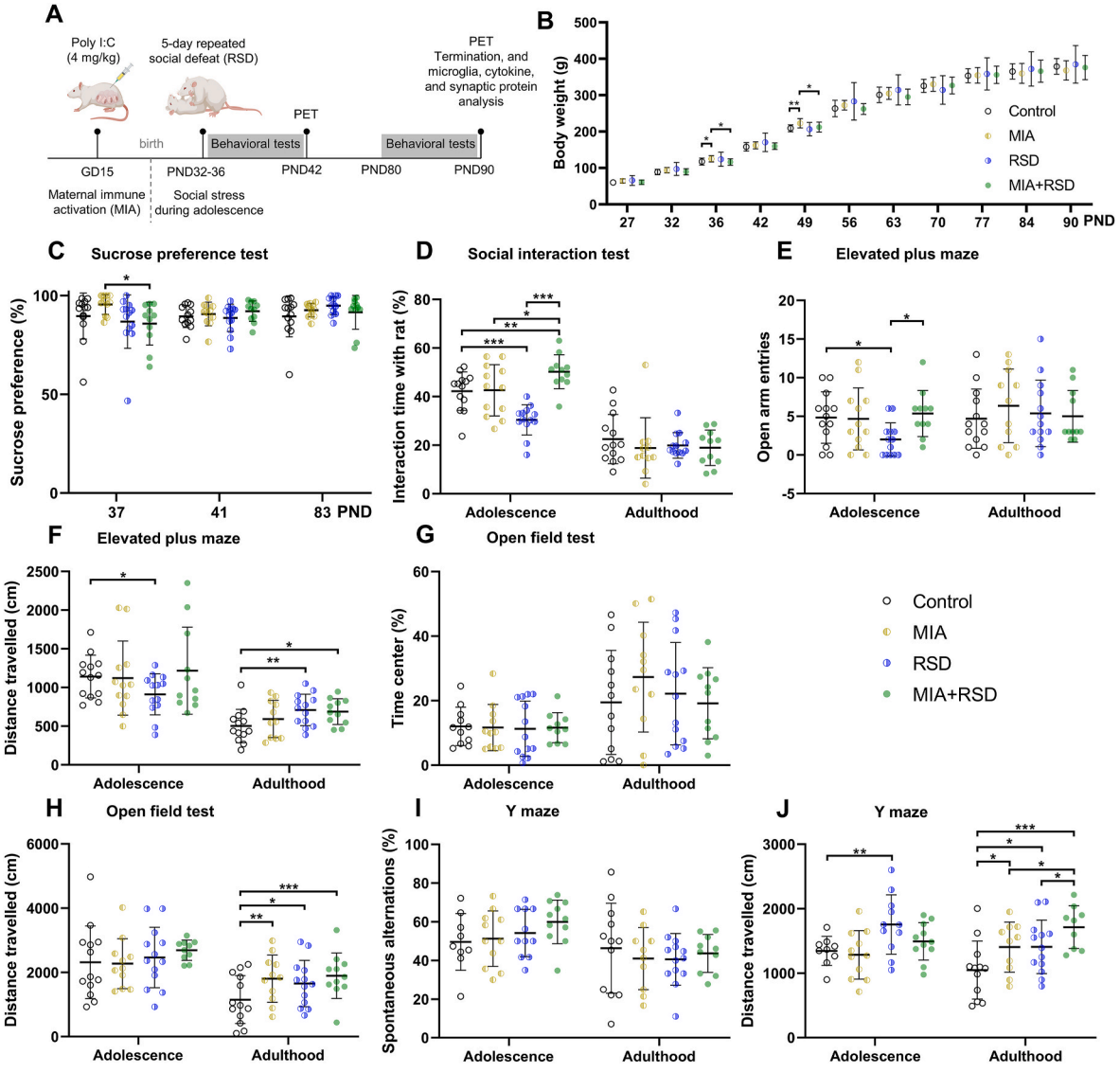

Fig. 1. Study design, bodyweight, anhedonia, social behavior, anxiety, locomotion and working memory in control, MIA, RSD, and MIA + RSD rats. (A) Study design. Pregnant dams were intravenously injected with either saline or poly I:C on gestational day (GD) 15. A 5-day repeated social defeat (RSD) protocol was performed between PND32-36. Behavioral experiments were conducted during adolescence (PND36-42) and adulthood (PND80-90). Blood samples were collected before each [^{11}C]PBR28 PET scan (PND42, 90). Brains were collected for Iba1 staining and cytokine and synaptic protein analysis on PND90. All the offspring males were exposed to all the behavioral experiments and PET scans. (B) Bodyweight. (C) Anhedonia in the sucrose preference test. (D) Social behavior in the social interaction test. (E, G) Anxiety in the elevated plus maze and the open-field test. I. Working memory in the Y-maze. (F, H, J) Locomotion. N = 11–13 rats per group. Data is presented as mean ± SD. GEE was performed using 'MIA', 'RSD' and 'time' as factors for longitudinal statistical analyses. Statistically significant differences between groups are indicated by asterisks: *p < 0.05, **p < 0.01, ***p < 0.001. Significant differences between time points and direct comparisons between MIA and RSD are not shown.

3.4. MIA overcompensated the RSD induced reduction in social interaction

To test social behavior, we quantified the preference of the rats for interacting with another rat (Fig. 1D). We observed a main effect of MIA ($W = 4.5$, $df = 1$, $p = 0.035$) and of time ($W = 191$, $df = 1$, $p < 0.001$), but not of RSD ($W = 0.83$, $df = 1$, $p = 0.364$). We also observed significant MIA*RSD ($W = 9.0$, $df = 1$, $p = 0.003$), and MIA*Time ($W = 16.2$, $df = 1$, $p < 0.001$) interactions. During adolescence, the RSD rats spent less time interacting with another rat than controls (−28%, $W = 261$, $df = 7$, $p < 0.001$). The opposite effect was observed in rats exposed to the combination of MIA and RSD, as the pairwise comparison ($W = 261$, $df = 7$, $p < 0.001$) revealed that their time interacting with another rat was significantly higher than that of control (+19%, $p = 0.006$), MIA (+18%, $p = 0.032$) and RSD rats (+67%, $p = <0.001$). These differences were not observed at adulthood anymore.

3.5. RSD increased anxiety-like behavior in the EPM

The EPM was used to assess anxiety-like behavior (Fig. 1E) and locomotion (Fig. 1F).

During adolescence, pairwise analysis revealed ($W = 20.6$, $df = 7$, $p = 0.004$) that RSD rats had a lower number of open arm entries compared to control (−58%, $p = 0.007$), and MIA + RSD rats (−62%, $p = 0.001$) (Fig. 1E). These differences were not observed in adulthood.

We observed a main effect of MIA ($W = 7.3$, $df = 1$, $p = 0.007$) and time ($W = 40.8$, $df = 1$, $p = 0.001$) but not of RSD ($W = 3.1$, $df = 1$, $p = 0.079$) on the time spent in closed arms. RSD rats spent significantly more time in the closed arms than MIA + RSD rats (+16%, $W = 63.2$, $df = 7$, $p = 0.014$) in adolescence but not adulthood (Supplementary Fig. S2A). We observed no significant difference between groups in the time spent in the open arm at any time point (Supplementary Fig. S2B). We observed a main effect of MIA ($W = 4.9$, $df = 1$, $p = 0.027$), RSD ($W = 10.1$, $df = 1$, $p = 0.001$), and time ($W = 63.2$, $df = 1$, $p < 0.001$) on the time spent in the center of the EPM (Supplementary Fig. S2C). In adolescence, pairwise comparison ($W = 154$, $df = 7$, $p < 0.001$) revealed that RSD rats (−65%, $p < 0.001$) spent significantly less time in the center of the EPM than controls and MIA + RSD rats spent significantly less time in the center of the EPM than control (−67%, $p < 0.001$) and MIA rats (−72%, $p < 0.001$). In adulthood, MIA rats spent significantly more time in the center of the EPM than controls (+59%, $p = 0.047$).

We observed a main effect of time on the distance traveled ($W = 64.1$, $df = 1$, $p < 0.001$) (Fig. 1F). The pairwise comparison ($W = 82.6$, $df = 7$, $p < 0.001$) revealed that during adolescence, RSD rats traveled significantly less than control rats (−20%, $p = 0.025$). At adulthood, RSD rats (+41%, 0.009) and RSD + MIA rats (+37%, 0.013) traveled significantly more than control animals.

3.6. RSD and MIA increased locomotion during adulthood in the OFT

The percentage of time spent in the center of the arena and the total distance traveled in the OFT were used to assess anxiety-like behavior (Fig. 1G) and locomotion (Fig. 1H), respectively. We observed no differences in time spent in the center, or in the total number of center entries between groups at any time point. In adulthood, pairwise analysis ($W = 64.3$, $df = 7$, $p < 0.001$) revealed that MIA (+105%, $p = 0.002$), RSD (+82%, $p = 0.026$) and RSD + MIA rats (+115%, $p < 0.001$) traveled significantly more than control rats.

3.7. RSD and MIA increased locomotion during adulthood in the Y-maze

Spontaneous alternations and distance traveled in the Y-maze were measured to assess working memory (Fig. 1I) and locomotion (Fig. 1J) respectively. We observed no differences in spontaneous alternations between groups at any time point. We observed a main effect of RSD ($W = 14.9$, $df = 1$, $p < 0.001$) on distance traveled. In adolescence, RSD rats traveled significantly more than control (+30%, $W = 25.1$, $df = 7$, $p = 0.006$). Pairwise comparisons ($W = 25.1$, $df = 1$, $p < 0.001$) revealed that adult RSD (+34%, $p = 0.030$), MIA (+34%, $p = 0.036$) and RSD + MIA rats (+64%, $p < 0.001$) traveled significantly more than controls. Furthermore, adult rats exposed to the combination of MIA + RSD traveled significantly more than rats exposed to RSD (+22%, $p = 0.043$) or MIA (+22%, $p = 0.047$) alone.

3.8. RSD reduced the MIA-induced increase in plasma corticosterone levels during adolescence

To determine the effect of MIA and RSD on the stress axis, serum concentrations of corticosterone were measured 1 week after exposure to adolescent RSD and in adulthood (Supplementary Fig. S3). We observed a MIA*Time interaction ($W = 7.1$, $df = 1$, $p = 0.008$) in corticosterone levels. Adolescent MIA rats had significantly higher corticosterone levels than controls (+63%, $W = 46.9$, $df = 7$, $p = 0.019$). Adulthood MIA rats had a significantly lower corticosterone levels than control rats (−40%, $W = 46.9$, $df = 7$, $p = 0.039$). These differences were not observed in MIA + RSD rats.

3.9. The combination of MIA and RSD induced a long-lasting increase in glial reactivity in the whole brain

To determine if the MIA- and RSD-induced behavioral changes were associated with changes in glial reactivity to the stressors, [^{11}C]PBR28 PET was performed during adolescence and adulthood (Fig. 2, Table 1). A main effect of time ($W = 154$, $df = 1$, $p = 0.001$) and MIA ($W = 21.0$, $df = 1$, $p < 0.001$) was observed on whole brain tracer uptake (Fig. 2A). Comparison with control animals during adolescence showed that MIA induced a significant increase in tracer uptake in all the brain regions measured (Fig. 2, Table 1), except for the amygdala. In adolescence, RSD induced a significant increase in tracer uptake in the whole brain (Fig. 2, Table 1), except for the amygdala, basal ganglia, insular and temporal cortices, forebrain, nucleus accumbens, and thalamus as compared to control rats. Adolescent rats exposed to the combination of MIA and RSD had a significantly higher tracer uptake than controls in all the brain regions measured (Fig. 2, Table 1), except for the amygdala and insular cortex. No difference between the RSD, MIA and MIA + RSD group were observed in adolescence.

In adulthood, MIA rats had a significantly higher tracer uptake in the brainstem (+17%, $W = 153$, $df = 7$, $p = 0.004$), cerebellum (+12%, $W = 319$, $df = 7$, $p = 0.028$), temporal cortex (+12%, $W = 20.1$, $df = 7$, $p = 0.023$), and hippocampus (+16%, $W = 201$, $df = 7$, $p = 0.017$) than controls. Pairwise comparisons ($W = 250$, $df = 7$, $p < 0.001$) showed that adult RSD rats had similar tracer uptake as controls in all brain regions. Rats exposed to the combination of MIA and RSD had a significantly enhanced tracer uptake in the whole brain as compared to control (+12%, $p = 0.003$) and RSD rats (+16%, $p < 0.001$), despite tracer uptake in several cortical areas being not significantly different between group (Fig. 2, Table 1). We observed no significant differences in tracer uptake between MIA and MIA + RSD rats.

3.10. The combination of MIA and RSD increased microglial cell density in the frontal cortex during adulthood

To further investigate the effect of MIA and RSD on microglia, the effect on microglia cell density was determined in the frontal and parietal cortices by counting the number of Iba1-positive cells (Fig. 3A). In adulthood, one-way ANOVA ($F(3, 16) = 7.3$), $p = 0.002$) revealed that the density of microglial cells in the frontal cortex (Fig. 3B) was significantly higher in the rats exposed to the combination of MIA and RSD than in control (+39%, $p = 0.007$), MIA (+34%, $p = 0.015$), and RSD rats (+41%, $p = 0.009$). In line with the increased microglial cell density, a significant decrease in the nearest neighbor distance between microglia was found (one-way ANOVA, $F(3, 16) = 6.9$, $p = 0.003$) in the

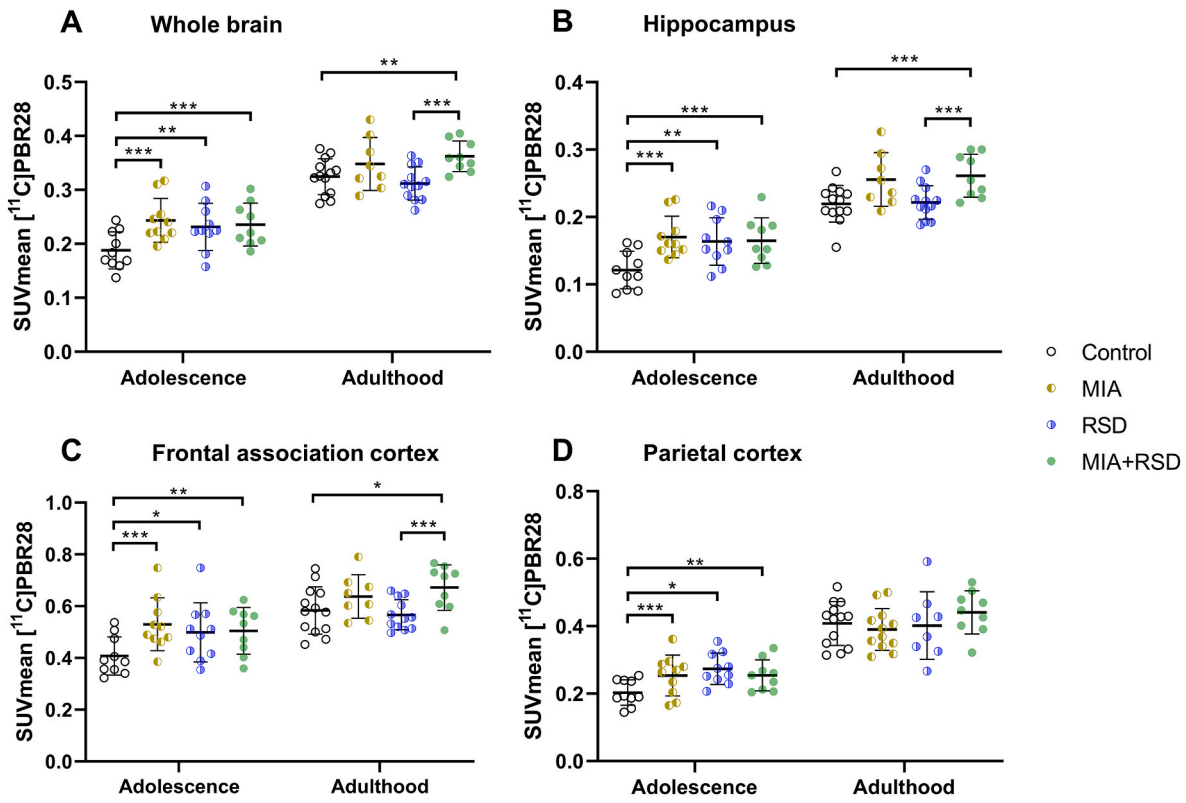

Fig. 2. TSPO expression level, associated with microglial reactivity, in the whole brain (A), Hippocampus (B), Frontal association cortex (C), and Parietal cortex (D) of control, MIA, RSD, and MIA + RSD rats as measure with [^{11}C]PBR28 PET. N = 8–13 per group. Graphs represent mean ± SD. GEE was performed using 'MIA', 'RSD' and 'time' as factors for longitudinal statistical analyses. Statistically significant differences between groups are indicated by asterisks: *p < 0.05, **p < 0.01, ***p < 0.001. Significant differences between time points and direct comparisons between MIA and RSD are not shown.

frontal cortex of the rats exposed to the combination of MIA and RSD, as compared to control (−13%, p = 0.013), MIA (−12%, p = 0.016), and RSD rats (−14%, p = 0.009) (Fig. 3D). In line with the PET data, we observed no difference in microglial density (Fig. 3C) and nearest neighbor distance (Fig. 3E) in the parietal cortex.

3.11. MIA increased IL-1β and RSD reduces IL-10 levels in the hippocampus during adulthood

IL-1β (Fig. 4A, B) and IL-10 (Fig. 4C, D) protein concentrations were measured in the hippocampus and frontal cortex with an ELISA as representative pro- and anti-inflammatory cytokines, respectively. In adulthood, one-way ANOVA ($F(3, 38) = 12.9$, $p < 0.001$) revealed that the IL-1β levels in the hippocampus were significantly higher in rats exposed to MIA, as compared to controls (+41%, $p < 0.001$) (Fig. 4A). The IL-1β levels in the hippocampus were also significantly higher in rats exposed to MIA + RSD, as compared to control (+27%, $p < 0.02$), and RSD rats (+36%, p = 0.003) (Fig. 4A). In adulthood, one-way ANOVA ($F(3, 38) = 5.6$, $p = 0.003$) revealed that the IL-10 levels in the hippocampus were significantly lower in rats exposed to RSD, as compared to control (−27%, p = 0.004), and MIA + RSD rats (−32%, p = 0.009) (Fig. 4C). IL-1β and IL-10 levels in the frontal cortex were similar in all groups (Fig. 4B, D).

3.12. MIA and RSD increased synaptophysin in the frontal cortex in adulthood

Synaptophysin (Fig. 5A, B) and SNAP25 (Fig. 5C, D) proteins were measured in the hippocampus and frontal cortex with ELISA. In adulthood, one-way ANOVA ($F(3, 37) = 4.3$, $p = 0.011$) revealed that the synaptophysin levels in the hippocampus were significantly higher in rats exposed to MIA, as compared to MIA + RSD rats (+42%, p = 0.048) (Fig. 5A). In the frontal cortex, one-way ANOVA ($F(3, 35) = 5.2$, $p = 0.004$) revealed that synaptophysin levels were significantly higher in the rats exposed to MIA (+64%, p = 0.0034) or RSD (+45%, p = 0.035), as compared to controls (Fig. 5B). SNAP25 levels were similar in all groups (Fig. 5C, D).

4. Discussion

In this study, we aimed to test whether exposure to maternal infection, social adversity trauma during adolescence and the combination of these factors can affect glial activity and density, synaptic density, and behavior. Although other studies have already investigated the dual hit hypothesis in rats, this study offers several new aspects that discriminate it from others. In particular, the combination of maternal infection with a specific second hit that consisted of exposing rats to social adversity during adolescence (while other studies used other stressors). Social adversity has the advantage of mimicking social stress that we experience as humans, and which is a known risk factor for psychiatric disorders. Furthermore, our study used PET imaging to longitudinally measure TSPO expression levels in the brain of rats exposed to these two stressors. The advantage of this methods is that it allows to use the same rats in adolescence and adulthood. To the best of our knowledge, this study is also the first one showing synaptic marker changes in rats exposed to the combination of these two stressors and observing a mutually protective effect of prenatal infection and adolescent social adversity on social behavior and anxiety.

More specifically, the present findings, summarized in Fig. 6, demonstrate that prenatal infection and social adversity during adolescence can be mutually protective. Maternal infection prevented short-term RSD-induced anxiety and reduced social behavior. Each hit alone or their combination induced hyperactivity in adulthood. Longitudinal PET imaging and Iba1 staining revealed a transient increase in

Table 1
[^{11}C]-PBR28 PET: tracer uptake in the brain of control, RSD, MIA, and MIA + RSD animals in adolescence and adulthood. Tracer uptake (SUV) is presented for different brain areas. Data are shown as mean ± SD. Statistically significant differences compared to control animals on the same day are indicated with an asterisk: *p < 0.05; **p < 0.01, ***p < 0.001. Statistically significant differences between RSD and MIA + RSD animals on the same day are indicated with a $^{\$}$: $^{\$}$p < 0.05; $^{\$\$}$p < 0.01, $^{\$\$\$}$p < 0.001. We observed no significant differences between MIA and MIA + RSD. Direct statistical comparisons between MIA and RSD are not shown.

Brain regions	Adolescence (PND42)				Adulthood (PND90)			
	Control	MIA	RSD	MIA + RSD	Control	MIA	RSD	MIA + RSD
Amygdala	0.193 ± 0.011	0.214 ± 0.008	0.220 ± 0.012	0.224 ± 0.014	0.230 ± 0.011	0.237 ± 0.014	0.220 ± 0.010	0.256 ± 0.019
Basal ganglia	0.127 ± 0.009	**0.156 ± 0.008***	0.148 ± 0.008	**0.156 ± 0.008***	0.187 ± 0.007	0.198 ± 0.006	0.189 ± 0.008	**0.221 ± 0.014***
Brainstem	0.178 ± 0.011	**0.218 ± 0.011***	**0.219 ± 0.010****	**0.220 ± 0.010*****	0.288 ± 0.008	**0.337 ± 0.015****	0.275 ± 0.008	**0.328 ± 0.015***$$**
Cerebellum	0.218 ± 0.014	**0.286 ± 0.016*****	**0.279 ± 0.018****	**0.278 ± 0.018*****	0.449 ± 0.012	**0.501 ± 0.020***	0.423 ± 0.017	**0.503 ± 0.019***$$**
Corpus Callosum	0.119 ± 0.008	**0.175 ± 0.010*****	**0.162 ± 0.012*****	**0.158 ± 0.010*****	0.268 ± 0.011	0.284 ± 0.020	0.259 ± 0.015	**0.301 ± 0.013***$**
Cortex Entorhinal	0.221 ± 0.014	**0.270 ± 0.009***	**0.264 ± 0.014***	**0.274 ± 0.018***	0.256 ± 0.010	0.284 ± 0.014	0.246 ± 0.009	0.283 ± 0.020
Cortex Frontal	0.261 ± 0.015	**0.346 ± 0.022*****	**0.324 ± 0.023***	**0.324 ± 0.016****	0.425 ± 0.015	0.441 ± 0.029	0.407 ± 0.017	0.467 ± 0.019$^{\$}$
Cortex Frontal Association	0.408 ± 0.022	**0.530 ± 0.031*****	**0.498 ± 0.034***	**0.504 ± 0.028****	0.584 ± 0.024	0.637 ± 0.028	0.567 ± 0.016	**0.672 ± 0.028***$$$**
Cortex Insular	0.257 ± 0.015	**0.309 ± 0.017***	0.282 ± 0.016	0.299 ± 0.016	0.278 ± 0.007	0.301 ± 0.010	0.284 ± 0.009	0.293 ± 0.017
Cortex Medial Prefrontal	0.176 ± 0.012	**0.258 ± 0.019*****	**0.231 ± 0.017*****	**0.238 ± 0.015*****	0.302 ± 0.014	0.338 ± 0.026	0.293 ± 0.021	**0.352 ± 0.013****$**
Cortex Occipital	0.180 ± 0.013	**0.251 ± 0.012*****	**0.238 ± 0.020***	**0.240 ± 0.018****	0.436 ± 0.025	0.436 ± 0.037	0.409 ± 0.015	0.468 ± 0.033
Cortex Orbitofrontal	0.291 ± 0.017	**0.382 ± 0.029****	**0.358 ± 0.022***	**0.369 ± 0.018****	0.375 ± 0.014	0.421 ± 0.022	0.371 ± 0.014	**0.436 ± 0.019***$$**
Cortex Parietal	0.203 ± 0.011	**0.274 ± 0.014*****	**0.254 ± 0.018***	**0.254 ± 0.014****	0.408 ± 0.017	0.402 ± 0.033	0.390 ± 0.017	0.441 ± 0.020
Cortex Temporal	0.249 ± 0.014	**0.301 ± 0.014***	0.289 ± 0.016	**0.301 ± 0.018***	0.285 ± 0.009	**0.320 ± 0.012***	0.282 ± 0.009	**0.322 ± 0.016***$**
Forebrain	0.134 ± 0.008	**0.172 ± 0.011*****	0.154 ± 0.009	**0.175 ± 0.008*****	0.237 ± 0.007	0.253 ± 0.11	0.225 ± 0.009	**0.280 ± 0.008***$$$**
Hippocampus	0.121 ± 0.008	**0.170 ± 0.009*****	**0.164 ± 0.011*****	**0.165 ± 0.010*****	0.220 ± 0.007	**0.256 ± 0.013****	0.222 ± 0.007	**0.261 ± 0.010****$$$**
Midbrain	0.114 ± 0.007	**0.159 ± 0.010*****	**0.144 ± 0.008****	**0.151 ± 0.010****	0.266 ± 0.010	0.297 ± 0.014	0.254 ± 0.009	**0.300 ± 0.014***$$**
Nucleus accumbens	0.168 ± 0.010	**0.216 ± 0.015****	0.199 ± 0.014	**0.207 ± 0.017***	0.230 ± 0.013	0.254 ± 0.016	0.211 ± 0.009	0.251 ± 0.015$^{\$}$
Striatum	0.116 ± 0.007	**0.157 ± 0.008*****	**0.143 ± 0.009***	**0.145 ± 0.008****	0.199 ± 0.007	0.216 ± 0.007	0.194 ± 0.007	0.220 ± 0.009$^{\$}$
Thalamus	0.137 ± 0.008	**0.175 ± 0.011****	0.157 ± 0.009	**0.179 ± 0.008*****	0.244 ± 0.008	0.259 ± 0.016	0.229 ± 0.006	**0.288 ± 0.017***$$**
Whole brain	0.188 ± 0.010	**0.244 ± 0.016*****	**0.231 ± 0.009****	**0.236 ± 0.013****	0.325 ± 0.009	0.348 ± 0.016	0.312 ± 0.009	**0.362 ± 0.009****$$** $

microglial cell density and reactivity to the stressors in adolescent rats exposed to MIA or RSD alone, whereas their combination induced a persistent increase until adulthood. Furthermore, prenatal infection increased proinflammatory cytokine IL-1β levels, whereas social adversity reduced anti-inflammatory cytokine IL-10 levels in the hippocampus during adulthood. This reduction in IL-10 levels was not observed in rats exposed to both MIA and RSD. Our study revealed that the increased synaptic markers in the frontal cortex of adult rats exposed to maternal infection or adolescent social adversity was absent in rats exposed to the combination of these stressors.

RSD and MIA mimic some of the behavioral effects of bullying in humans and maternal infection, respectively (Björkqvist, 2001; Brown and Meyer, 2018; Careaga et al., 2017). In accordance with other studies (Calpe-López et al., 2022; Shimizu et al., 2020; Warren et al., 2014), we found that within a week after the adolescent RSD protocol, rats displayed a reduction in social behavior and increased anxiety as indicated by a reduced time spent playing with another rat and a reduced number of entries in the open arm of the EPM, respectively. These effects were not observed in MIA offspring exposed to the RSD protocol, suggesting a protective effect of MIA. The literature shows various effects of maternal infection during pregnancy as being either protective against stressors during adolescence or synergistically detrimental (reviewed in Guerrin et al., 2021). One study observed a protective effect of MIA on social isolation associated with higher levels of hippocampal oxytocin, a hormone capable of reducing anxiety and social avoidance notably by modulating the glucocorticoid response (Engelmann et al., 2004; Goh et al., 2020; Yoon and Kim, 2020). Perhaps similar oxytocin changes have occurred in our study, which could explain the mutual protective effect of MIA and RSD on anxiety, social behavior, and plasma corticosterone levels. The protective effect of MIA aligns with the suggestion that a certain level of adversity in humans can increase resilience (Seery et al., 2013). As group housing can counteract the effect of social adversity, rats exposed to social adversity during adolescence were socially isolated during the RSD protocol and until the first PET scan (6 days later). Thus, it should be emphasized that our findings are the result of the combined effect of RSD and social isolation, which is a plausible combination of stressors that is often observed in humans as well.

In accordance with the literature, we observed that prenatal infection (Desbonnet et al., 2022; Gzielo et al., 2021; Howland et al., 2012; Li et al., 2018; Zhu et al., 2014), social adversity during adolescence or their combination (Burke et al., 2013, 2011; Watt et al., 2009; Giovanoli et al., 2013, 2016a,b) induced hyperlocomotion in adulthood in the OFT, EPM, and Y-maze. Hyperlocomotion has been attributed to alterations in the mesolimbic dopaminergic pathway (Fabricius et al., 2011).

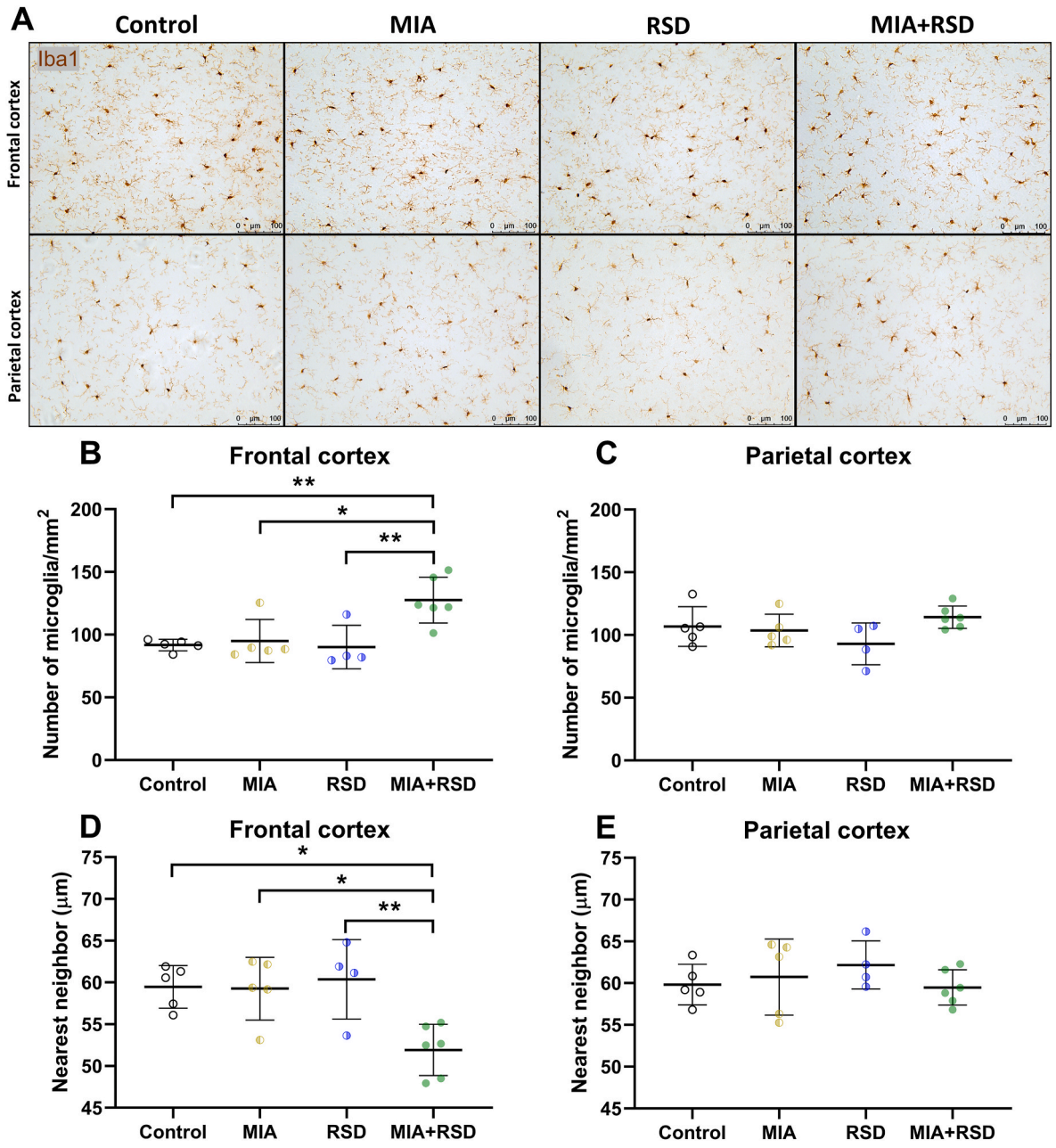

Fig. 3. Density of microglia and nearest neighbor distance in adult control, MIA, RSD, and MIA + RSD rats. Representative Iba1 staining of microglia in the frontal and parietal cortex of control, MIA, RSD, and MIA + RSD rats (A). Number of Iba1-positive cells/mm^2 in the frontal (B) and parietal cortex (C). Nearest neighbor distance analysis in the frontal (D) and parietal cortices (E). n = 4–6 rats per group. Graphs represent mean ± SD. A one-way ANOVA, followed by a Tukey's multiple comparisons post hoc test was performed. Statistically significant differences between groups are indicated by asterisks: *p < 0.05, **p < 0.01. Direct comparisons between MIA and RSD are not shown.

Our results are in line with clinical studies showing hyperactivity and psychomotor symptoms in patients with schizophrenia (Sams-Dodd et al., 1997; Sano et al., 2012) and ASD (Lai et al., 2019).

Rats exposed to MIA or RSD during adolescence, or the combination of both stressors showed no significant changes in social behavior, anhedonia, anxiety and working memory in the social interaction test, SPT, OFT, EPM and Y-maze during adulthood. Our findings contrast with other studies that observed adult MIA offspring displaying reduced social behavior (Andoh et al., 2019; Mattei et al., 2017; Mueller et al., 2021; Pendyala et al., 2017; Van Den Eynde et al., 2014; Wang et al., 2019; Zhu et al., 2014), increased anxiety in the OFT and EPM (Van Den Eynde et al., 2014; Wang et al., 2019) and reduced working memory in the Y-maze (Mueller et al., 2021; Richetto et al., 2013). Differences in the timing and dose of the maternal infection may explain these differences as these parameters have been shown to critically determine the patterns of behavioral abnormalities displayed in the offspring at adult age (Meyer et al., 2006, 2008). In contrast to our study, some studies observed that social adversity during adolescence resulted in a reduction in sucrose preference in the SPT, increased anxiety, and reduced social preference (Buwalda et al., 2013; Guerrin et al., 2023; Iñiguez et al., 2014; Parise et al., 2020; Resende et al., 2016; Warren et al., 2013, 2014), while others observed no long-lasting effects on these parameters (Buwalda et al., 2013; Mouri et al., 2018). One of the differences in the social interaction test between our and other studies is that we measured the willingness of the rat to socially interact, while other studies measured social avoidance by measuring the willingness of the

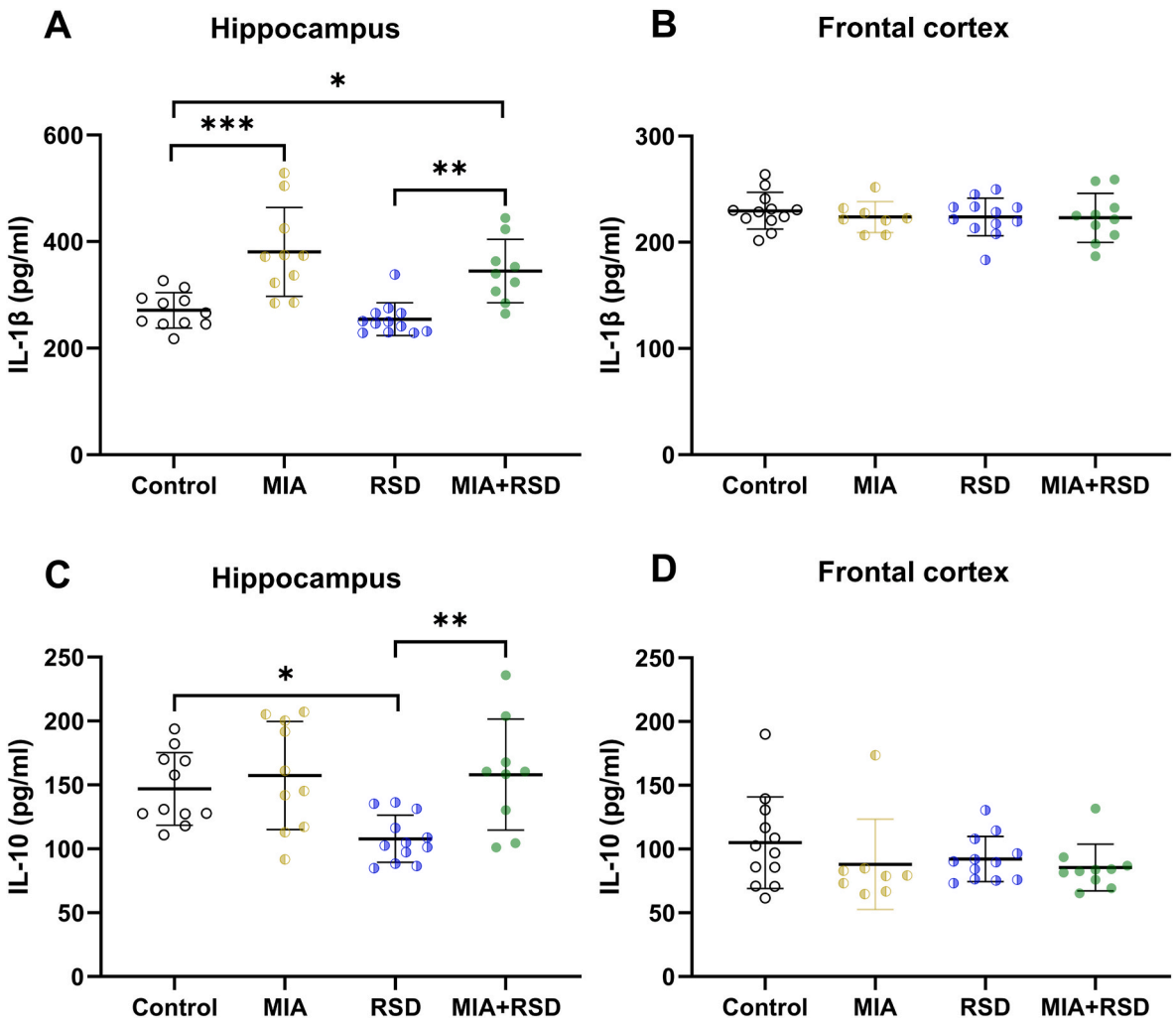

Fig. 4. IL-1β and IL-10 protein levels in the brain of adult control, MIA, RSD, and MIA + RSD rats. IL-1 β protein levels in the hippocampus (A) and the frontal cortex (**B**). IL-10 protein levels in the hippocampus (C) and the frontal cortex (D). N = 8–12 per group. Graphs represent mean ± SD. A one-way ANOVA, followed by a Tukey's multiple comparisons post hoc test was performed. Statistically significant differences between groups are indicated by asterisks: *p < 0.05, **p < 0.01, ***p < 0.001. Direct comparisons between MIA and RSD are not shown.

experimental rodent to interact with a rodent by which it was defeated in the RSD protocol. Future studies should perform other behavioral tests to explore more thoroughly the behavioral pattern induced by the combination of maternal infection and social adversity during adolescence. These tests could include marble burying or prepulse inhibition tests as they represent behavior related to ASD and schizophrenia, respectively.

Deviant activity and density of microglia has been associated with several neurodevelopmental and affective disorders. To the best of our knowledge, this is the first longitudinal PET imaging study measuring TSPO levels in rats exposed to MIA and RSD. Our longitudinal within-group comparisons using [^{11}C]PBR28 PET revealed increased TSPO levels, a protein associated with microglial reactivity, in the whole brain of adolescent rats exposed to MIA, adolescent RSD, or a combination of both stressors. While glial reactivity was transient in rats exposed to adolescent RSD, MIA offspring still showed higher PET tracer uptake in the hippocampus, temporal cortex, cerebellum, and brainstem in adulthood. Only the adult rats exposed to the combination of MIA and adolescent RSD showed a long-lasting increase microglial reactivity in the whole brain. Furthermore, the number of Iba1-positive cells was higher in the frontal, but not parietal, cortex of adult rats exposed to the combination of both stressors, as compared to healthy controls or rats exposed to a single stressor. These results suggest that the combination of two stressors, as opposed to a single stressor, has long-term effects on microglia reactivity and density. Our results are consistent with clinical studies observing increased microglial reactivity and density in the brain of patients with ASD (Morgan et al., 2010; Tetreault et al., 2012). Furthermore, our TSPO PET results have translational value as TSPO tracers can also be used in patients and imaging studies have observed increased proinflammatory states and TSPO upregulation in the brain of people suffering from major depressive disorder and schizophrenia (Gritti et al., 2021; Kim and Won, 2017; Marques et al., 2019). However, PET tracers for imaging of TSPO have limitations, as TSPO is not specifically expressed on microglia cells but also on other cells of the central nervous system, such as astrocytes and endothelial cells (Janssen et al., 2018). In addition, its overexpression does not discriminate between a change in microglia density or reactivity as in both cases an increase in TSPO may be induced. Anesthesia may be a confounding factor in PET imaging studies, as it could potentially alter radiotracer pharmacokinetics and interact with neurotransmitter systems. A previous study noted a 26% reduction in PBR28 TSPO binding in healthy human subjects under propofol anesthesia (Hines et al., 2013). Although we used a different anesthetic, isoflurane, similar effects are plausible in our study. In mice, prolonged isoflurane anesthesia (>2 h) indeed reduced TSPO binding in brown adipose tissue as compared to short-term anesthesia (<2 h) (Lee et al., 2022). Despite that the study was not conducted in the

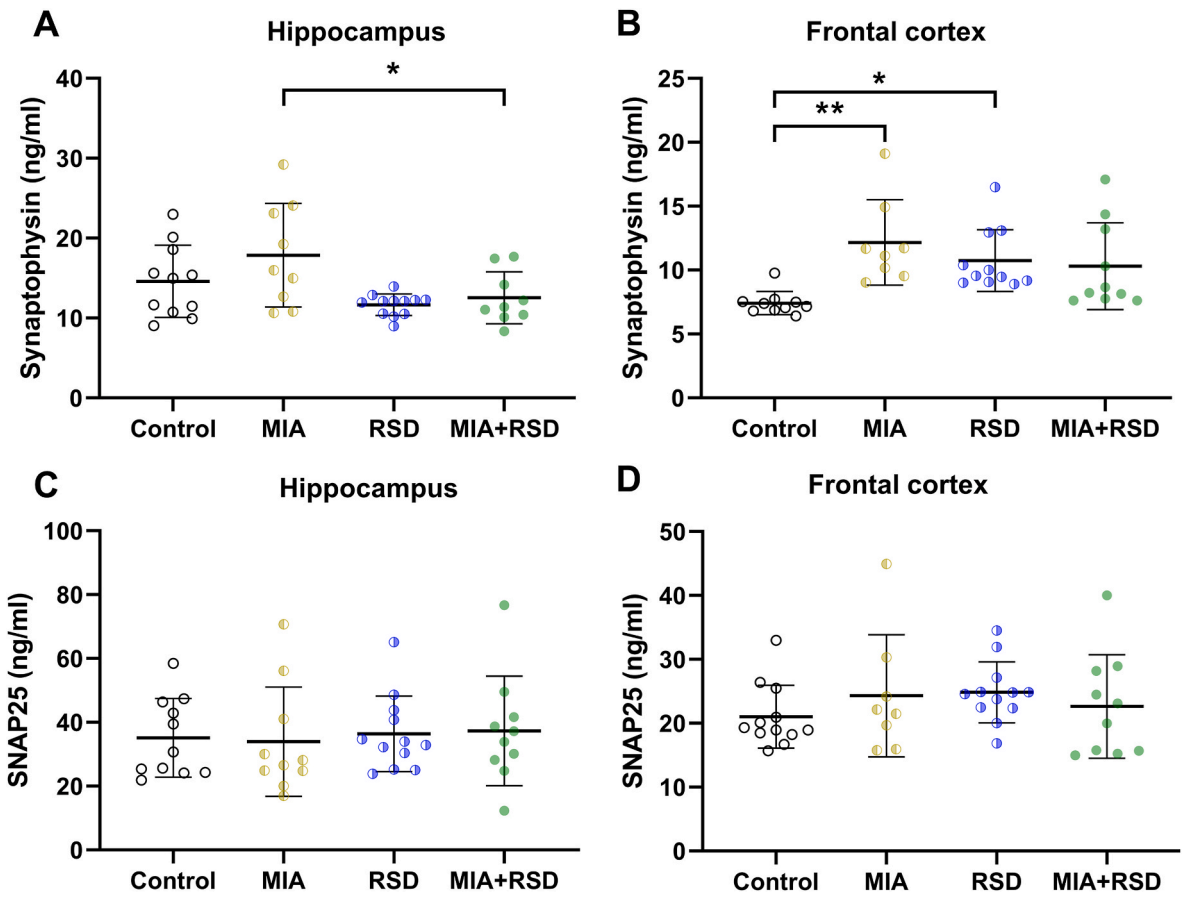

Fig. 5. Synaptophysin and SNAP25 in adult control, MIA, RSD, and MIA + RSD rats. Synaptophysin protein levels in the hippocampus (A) and the frontal cortex (B). SNAP25 protein levels in the hippocampus (C) and the frontal cortex (D). N = 8–12 per group. Graphs represent mean ± SD. A one-way ANOVA, followed by a Tukey's multiple comparisons post hoc test was performed. Statistically significant differences between groups are indicated by asterisks: *p < 0.05, **p < 0.01. Direct comparisons between MIA and RSD are not shown.

brain, it suggests that the short anesthesia duration (45 min) performed in our PET imaging likely has a minimal impact on TSPO uptake. Besides, any anesthesia-related effect in our study would likely result in an underestimation of the findings, if any. More importantly, anesthesia was applied uniformly across all groups, ensuring consistency in the effect of this potential confounder.

Our study also found that MIA, alone or combined with RSD, induced an increase in the proinflammatory cytokine IL-1β protein levels, while RSD alone reduced the anti-inflammatory cytokine IL-10 protein levels in the hippocampus during adulthood. However, no differences in cytokine levels in frontal cortex were observed. These interesting findings suggest that the increased TSPO levels and microglial density in the frontal cortex of adult rats exposed to MIA + RSD were not accompanied by release of IL-1β or IL-10, while in hippocampus they were. A possible explanation could be that the production of cytokines in stimulated microglia in frontal cortex was locally inhibited by activation of microglial cannabinoid receptors (Cabral and Marciano-Cabral, 2005). However, further investigation is needed to explain this apparent discrepancy.

In addition, these findings differ from some other studies that observed increased microglial numbers without changes in pro-inflammatory cytokines (Giovanoli et al., 2016a,b; Mattei et al., 2017; Purves-Tyson et al., 2019), or no changes in both microglia density and pro-inflammatory cytokine levels (Giovanoli et al., 2015). In contrast with our study, another research group observed that the combination of MIA and mild stressors during adolescence was necessary to induce a transient increase in microglial density and pro-inflammatory cytokines in the hippocampus and frontal cortex, while each hit alone did not induce such changes (Giovanoli et al., 2013, 2016a,b). These apparent differences can be explained by difference in experimental protocols, such as injected dose of poly I:C, timing of injection, stress models, and species variations.

As microglia are involved in neurodevelopmental processes such as synaptic pruning, we investigated potential changes in synaptic markers in adult rats (Germann et al., 2021; Kim et al., 2021; Koyama and Ikegaya, 2015; Lee et al., 2019). Unlike previous studies that explored synaptic markers in rats exposed to a single hit (Giovanoli et al., 2016a, b; Han et al., 2022), our study also explored synaptic markers in rats exposed to the combination of MIA and RSD. We observed that exposure to MIA and adolescent RSD alone increased synaptophysin levels in the frontal cortex but not hippocampus. Such an increase in synaptophysin was not observed if the two stressors were combined, perhaps because a higher number of microglia over a longer period of time could have counteracted the possible phagocytic dysfunction induced by a single hit alone, thus resulting in synaptophysin levels similar to controls. In support of this hypothesis, other studies observed an association between increased spine density and synaptic markers in MIA offspring with a reduction in CX3CR1 mRNA (involved in synaptic pruning) (Fernández de Cossío et al., 2017) and altered microglia-dependent synaptic engulfment (Andoh et al., 2019). Furthermore, another study observed that the phagocytic activity of hippocampal microglial cells was decreased in adult MIA offspring despite an increased density of Iba1-positive cells (Mattei et al., 2017). Maternal separation-induced higher spine density in the adolescent hippocampus was associated with an impaired phagocytic microglial ability (Dayananda et al., 2023). On the other hand, social defeat during adulthood was shown to increase

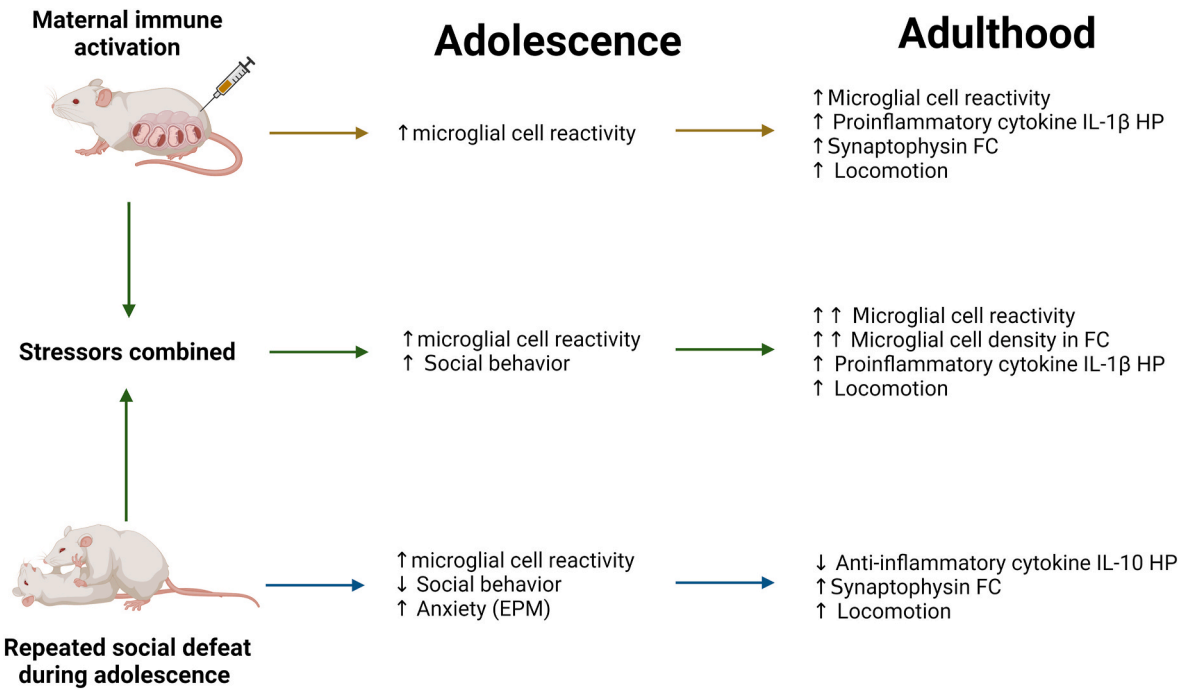

Fig. 6. Summary of the effect of MIA, RSD and their combination on behavior, microglia, brain cytokines and synaptic protein. Arrows up and down represent significant differences compared to control animals on the same day. ↔ = no significant changes, ↑ = increase, ↑↑ = synergistic increase, ↓ = decrease. EPM = elevated-plus maze, HP = hippocampus, FC = frontal cortex, IL-10 = interleukin 10, IL-1β = interleukin 1 β. Created with BioRender.com.

microglial phagocytic activity, thus resulting in a reduction in hippocampal PSD95 shortly after the stressor (Han et al., 2022). Furthermore, previous studies observed that RSD during adolescence induced an increase in spine density in the nucleus accumbens (Warren et al., 2014) and a reduction in stubby spines in the hippocampus without a change in the density of dendritic spines (Iñiguez et al., 2016), 24 h following the last stressor. Our results are in line with clinical studies that observed increased synaptic density in the brain of patients with ASD (Ebrahimi-Fakhari and Sahin, 2015; Gomot et al., 2008; Hutsler and Zhang, 2010; Yizhar et al., 2011). Unfortunately, we did not find clear correlations between behavior, immune system, and synaptic markers. Since we did not directly measure synaptic pruning, future studies should conduct an analysis of PSD95 engulfment in $CD68^{+}$ cells, a marker of phagocytic microglia.

Upstream mechanisms, such as synapse opsonization by the complement system or astrocyte-related synaptic pruning, may be modified by MIA and adolescent RSD, thus resulting in reduced detection of synapses eligible for phagocytose by microglia (Germann et al., 2021; Mattei et al., 2017). Although our design does not allow differentiation between reduced synaptic pruning or increased synaptogenesis and neurogenesis, we found that MIA, RSD or the combination of both did not modify SNAP25 levels in the adult hippocampus and frontal cortex. SNAP25 is involved in the structure and/or function of postsynaptic compartment and spine morphogenesis (Antonucci et al., 2016; Corradini et al., 2009), thus suggesting that spine morphogenesis was not modified by MIA and RSD in our study. This hypothesis, however, still awaits further confirmation.

These changes in synaptic density markers may explain some of the behavior observed in our study. While anxiety, depression and cognitive deficits in humans and animals are associated with a reduced synaptic density, we observed an increase in synaptophysin, a synaptic marker. This may explain why we did not observe such behavioral alterations in adult rats exposed to MIA, RSD during adolescence or their combination (Duric et al., 2013; Han et al., 2022; Zeng et al., 2012; Zhao et al., 2012). Furthermore, the increased synaptophysin in the frontal cortex observed in our study may partly explain the increased excitatory output that results in increased motor activity (Flores et al., 2016).

5. Conclusion

In conclusion, our study we examined the impact of maternal infection, adolescent social adversity, and their combination on glial activity, synaptic density, and behavior in rats. Our research uniquely combined maternal infection with adolescent social adversity, mirroring human social stress, and employed longitudinal PET imaging. We observed that prenatal infection and social adversity during adolescence can be mutually protective as maternal infection during pregnancy prevented the reduction in social behavior and the increase in anxiety induced by social adversity during adolescence. Furthermore, each stressor alone or in combination induced hyperlocomotion in adulthood. Prenatal maternal infection and social defeat induced a transient increase in the density of reactive microglia during adolescence, while their combination induced a long-lasting increase that remained until adulthood. Prenatal maternal infection and social adversity during adolescence also increased synaptic markers, in the frontal cortex, while sparing the hippocampus. The increase in synaptic markers was not observed in rats exposed to both stressors, perhaps due to the long-lasting increase in microglial cell density, likely accompanied by an increase in microglial synaptic pruning. Future studies are needed to further investigate these changes in synaptic pruning induced by maternal infection and social adversity during adolescence.

Credit author statement

Cyprien G. J. Guerrin: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – Original draft, Supervision. Kavya Prasad: Investigation, Writing – Review & Editing. Daniel A. Vazquez-Matias: Investigation, Writing – Review & Editing. Jing Zheng: Validation, Investigation. Maria Franquesa-Mullerat: Validation, Investigation. Lara Barazzuol: Methodology, Resources, Writing – Review & Editing. Janine Doorduin: Conceptualization, Writing – Review & Editing, Supervision. Erik F. J. de Vries: Conceptualization, Writing –

Review & Editing, Supervision.

Formatting of funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank Prof. Dr. Wia Baron and her co-workers and Jurgen Sijbesma for the experimental support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynstr.2023.100580.

References

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., Llorca, P.-M., Arango, C., Tortelli, A., de Haan, L., Velthorst, E., Bobes, J., Bernardo, M., Sanjuán, J., Santos, J.L., Arrojo, M., Del-Ben, C.M., Menezes, P.R., Selten, J.-P., Jones, P.B., Jongsma, H.E., Kirkbride, J.B., Rutten, B.P.F., van Os, J., Gayer-Anderson, C., Murray, R.M., Morgan, C., 2021. Synergistic effects of childhood adversity and polygenic risk in first-episode psychosis: the EU-GEI study. Psychol. Med. 1–9. https://doi.org/10.1017/S0033291721003664.

Andoh, M., Shibata, K., Okamoto, K., Onodera, J., Morishita, K., Miura, Y., Ikegaya, Y., Koyama, R., 2019. Exercise reverses behavioral and synaptic abnormalities after maternal inflammation. Cell Rep. 27 https://doi.org/10.1016/j.celrep.2019.05.015, 2817-2825.e5.

Antonucci, F., Corradini, I., Fossati, G., Tomasoni, R., Menna, E., Matteoli, M., 2016. SNAP-25, a known presynaptic protein with emerging postsynaptic functions. Front. Synaptic Neurosci. 8, 7. https://doi.org/10.3389/fnsyn.2016.00007.

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

Beckers, L., Ory, D., Geric, I., Declercq, L., Koole, M., Kassiou, M., Bormans, G., Baes, M., 2018. Increased expression of translocator protein (TSPO) marks pro-inflammatory microglia but does not predict neurodegeneration. Mol. Imag. Biol. 20, 94–102. https://doi.org/10.1007/s11307-017-1099-1.

Biswas, L., Farhan, F., Reilly, J., Bartholomew, C., Shu, X., 2018. TSPO ligands promote cholesterol efflux and suppress oxidative stress and inflammation in choroidal endothelial cells. Int. J. Mol. Sci. 19 https://doi.org/10.3390/ijms19123740.

Björkqvist, K., 2001. Social defeat as a stressor in humans. Physiol. Behav. 73, 435–442. https://doi.org/10.1016/S0031-9384(01)00490-5.

Brown, A.S., 2011. The environment and susceptibility to schizophrenia. Prog. Neurobiol. 93, 23–58. https://doi.org/10.1016/j.pneurobio.2010.09.003.

Brown, A.S., Meyer, U., 2018. Maternal immune activation and neuropsychiatric illness: a translational research perspective. Am. J. Psychiatr. https://doi.org/10.1176/appi.ajp.2018.17121311.

Burke, A.R., Forster, G.L., Novick, A.M., Roberts, C.L., Watt, M.J., 2013. Effects of adolescent social defeat on adult amphetamine-induced locomotion and corticoaccumbal dopamine release in male rats. Neuropharmacology 67, 359–369. https://doi.org/10.1016/j.neuropharm.2012.11.013.

Burke, A.R., Watt, M.J., Forster, G.L., 2011. Adolescent social defeat increases adult amphetamine conditioned place preference and alters D2 dopamine receptor expression. Neuroscience 197, 269–279. https://doi.org/10.1016/j.neuroscience.2011.09.008.

Buwalda, B., Stubbendorff, C., Zickert, N., Koolhaas, J.M., 2013. Adolescent social stress does not necessarily lead to a compromised adaptive capacity during adulthood: a study on the consequences of social stress in rats. Neuroscience 249, 258–270. https://doi.org/10.1016/j.neuroscience.2012.12.050.

Cabral, G.A., Marciano-Cabral, F., 2005. Cannabinoid receptors in microglia of the central nervous system: immune functional relevance. J. Leukoc. Biol. 78, 1192–1197. https://doi.org/10.1189/jlb.0405216.

Calpe-López, C., Martínez-Caballero, M.A., García-Pardo, M.P., Aguilar, M.A., 2022. Intermittent voluntary wheel running promotes resilience to the negative consequences of repeated social defeat in mice. Physiol. Behav. 254, 113916 https://doi.org/10.1016/j.physbeh.2022.113916.

Cardozo, P.L., de Lima, I.B.Q., Maciel, E.M.A., Silva, N.C., Dobransky, T., Ribeiro, F.M., 2019. Synaptic elimination in neurological disorders. Curr. Neuropharmacol. 17, 1071–1095. https://doi.org/10.2174/1570159X17666190603170511.

Careaga, M., Murai, T., Bauman, M.D., 2017. Maternal immune activation and autism spectrum disorder: from rodents to nonhuman and human primates. Biol. Psychiatr. 81, 391–401. https://doi.org/10.1016/j.biopsych.2016.10.020.

Corradini, I., Verderio, C., Sala, M., Wilson, M.C., Matteoli, M., 2009. SNAP-25 in neuropsychiatric disorders. Ann. N. Y. Acad. Sci. 1152, 93–99. https://doi.org/10.1111/j.1749-6632.2008.03995.x.

Dayananda, K.K., Ahmed, S., Wang, D., Polis, B., Islam, R., Kaffman, A., 2023. Early life stress impairs synaptic pruning in the developing hippocampus. Brain Behav. Immun. 107, 16–31. https://doi.org/10.1016/j.bbi.2022.09.014.

Debost, J.C.P.G., Larsen, J.T., Munk-Olsen, T., Mortensen, P.B., Meyer, U., Petersen, L., 2017. Joint effects of exposure to prenatal infection and peripubertal psychological trauma in schizophrenia. Schizophr. Bull. 43, 171–179. https://doi.org/10.1093/schbul/sbw083.

Desbonnet, L., Konkoth, A., Laighneach, A., McKernan, D., Holleran, L., McDonald, C., Morris, D.W., Donohoe, G., Kelly, J., 2022. Dual hit mouse model to examine the long-term effects of maternal immune activation and post-weaning social isolation on schizophrenia endophenotypes. Behav. Brain Res. 430, 113930 https://doi.org/10.1016/j.bbr.2022.113930.

Duric, V., Banasr, M., Stockmeier, C.A., Simen, A.A., Newton, S.S., Overholser, J.C., Jurjus, G.J., Dieter, L., Duman, R.S., 2013. Altered expression of synapse and glutamate related genes in post-mortem hippocampus of depressed subjects. Int. J. Neuropsychopharmacol. 16, 69–82. https://doi.org/10.1017/S1461145712000016.

Ebrahimi-Fakhari, D., Sahin, M., 2015. Autism and the synapse: emerging mechanisms and mechanism-based therapies. Curr. Opin. Neurol. 28, 91–102. https://doi.org/10.1097/WCO.0000000000000186.

Engelmann, M., Landgraf, R., Wotjak, C.T., 2004. The hypothalamic–neurohypophysial system regulates the hypothalamic–pituitary–adrenal axis under stress: an old concept revisited. Front. Neuroendocrinol. 25, 132–149. https://doi.org/10.1016/j.yfrne.2004.09.001.

Fabricius, K., Steiniger-Brach, B., Helboe, L., Fink-Jensen, A., Wörtwein, G., 2011. Socially isolated rats exhibit changes in dopamine homeostasis pertinent to schizophrenia. Int. J. Dev. Neurosci. 29, 347–350. https://doi.org/10.1016/j.ijdevneu.2010.09.003.

Fernández de Cossío, L., Guzmán, A., van der Veldt, S., Luheshi, G.N., 2017. Prenatal infection leads to ASD-like behavior and altered synaptic pruning in the mouse offspring. Brain Behav. Immun. 63, 88–98. https://doi.org/10.1016/j.bbi.2016.09.028.

Flores, G., Morales-Medina, J.C., Diaz, A., 2016. Neuronal and brain morphological changes in animal models of schizophrenia. Behav. Brain Res. 301, 190–203. https://doi.org/10.1016/j.bbr.2015.12.034.

Germann, M., Brederoo, S.G., Sommer, I.E.C., 2021. Abnormal synaptic pruning during adolescence underlying the development of psychotic disorders. Curr. Opin. Psychiatr. 34, 222–227. https://doi.org/10.1097/YCO.0000000000000696.

Giovanoli, S., Engler, H., Engler, A., Richetto, J., Feldon, J., Riva, M.A., Schedlowski, M., Meyer, U., 2016a. Preventive effects of minocycline in a neurodevelopmental two-hit model with relevance to schizophrenia. Transl. Psychiatry 6, e772–e779. https://doi.org/10.1038/tp.2016.38.

Giovanoli, S., Engler, H., Engler, A., Richetto, J., Voget, M., Willi, R., Winter, C., Riva, M. A., Mortensen, P.B., Schedlowski, M., Meyer, U., 2013. Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice. Science 339, 1100–1102. https://doi.org/10.1126/science.1228261.

Giovanoli, S., Notter, T., Richetto, J., Labouesse, M.A., Vuillermot, S., Riva, M.A., Meyer, U., 2015. Late prenatal immune activation causes hippocampal deficits in the absence of persistent inflammation across aging. J. Neuroinflammation 12, 221. https://doi.org/10.1186/s12974-015-0437-y.

Giovanoli, Sandra, Weber-Stadlbauer, U., Schedlowski, M., Meyer, U., Engler, H., 2016b. Prenatal immune activation causes hippocampal synaptic deficits in the absence of overt microglia anomalies. Brain Behav. Immun. 55, 25–38. https://doi.org/10.1016/j.bbi.2015.09.015.

Goh, J.Y., O'Sullivan, S.E., Shortall, S.E., Zordan, N., Piccinini, A.M., Potter, H.G., Fone, K.C.F., King, M.V., 2020. Gestational poly(I:C) attenuates, not exacerbates, the behavioral, cytokine and mTOR changes caused by isolation rearing in a rat 'dual-hit' model for neurodevelopmental disorders. Brain Behav. Immun. 89, 100–117. https://doi.org/10.1016/j.bbi.2020.05.076.

Gomot, M., Belmonte, M.K., Bullmore, E.T., Bernard, F.A., Baron-Cohen, S., 2008. Brain hyper-reactivity to auditory novel targets in children with high-functioning autism. Brain 131, 2479–2488. https://doi.org/10.1093/brain/awn172.

Gritti, D., Delvecchio, G., Ferro, A., Bressi, C., Brambilla, P., 2021. Neuroinflammation in major depressive disorder: a review of PET imaging studies examining the 18-kDa translocator protein. J. Affect. Disord. 292, 642–651. https://doi.org/10.1016/j.jad.2021.06.001.

Guerrin, C.G.J., Doorduin, J., Prasad, K., Vazquez-Matias, D.A., Barazzuol, L., de Vries, E. F.J., 2023. Social adversity during juvenile age but not adulthood increases susceptibility to an immune challenge later in life. Neurobiol. Stress 23, 100526. https://doi.org/10.1016/j.ynstr.2023.100526.

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. Neurosci. Biobehav. Rev. 131, 1150–1168. https://doi.org/10.1016/j.neubiorev.2021.10.025.

Gzielo, K., Potasiewicz, A., Litwa, E., Piotrowska, D., Popik, P., Nikiforuk, A., 2021. The effect of maternal immune activation on social play-induced ultrasonic vocalization in rats. Brain Sci. 11 https://doi.org/10.3390/brainsci11030344.

Han, Q.-Q., Shen, S.-Y., Chen, X.-R., Pilot, A., Liang, L.-F., Zhang, J.-R., Li, W.-H., Fu, Y., Le, J.-M., Chen, P.-Q., Yu, J., 2022. Minocycline alleviates abnormal microglial phagocytosis of synapses in a mouse model of depression. Neuropharmacology 220, 109249. https://doi.org/10.1016/j.neuropharm.2022.109249.

Herbert, M.R., 2010. Contributions of the environment and environmentally vulnerable physiology to autism spectrum disorders. Curr. Opin. Neurol. 23, 103–110. https://doi.org/10.1097/WCO.0b013e328336a01f.

Herrera-Rivero, M., Heneka, M.T., Papadopoulos, V., 2015. Translocator protein and new targets for neuroinflammation. Clin. Transl. Imaging 3, 391–402. https://doi.org/10.1007/s40336-015-0151-x.

Hines, C.S., Fujita, M., Zoghbi, S.S., Kim, J.S., Quezado, Z., Herscovitch, P., Miao, N., Ferraris Araneta, M.D., Morse, C., Pike, V.W., Labovsky, J., Innis, R.B., 2013. Propofol decreases in vivo binding of 11C-PBR28 to translocator protein (18 kDa) in the human brain. J. Nucl. Med. 54, 64–69. https://doi.org/10.2967/jnumed.112.106872.

Howes, O.D., McCutcheon, R., 2017. Inflammation and the neural diathesis-stress hypothesis of schizophrenia: a reconceptualization. Transl. Psychiatry 7, e1024-11. https://doi.org/10.1038/tp.2016.278.

Howland, J.G., Cazakoff, B.N., Zhang, Y., 2012. Altered object-in-place recognition memory, prepulse inhibition, and locomotor activity in the offspring of rats exposed to a viral mimetic during pregnancy. Neuroscience 201, 184–198. https://doi.org/10.1016/j.neuroscience.2011.11.011.

Hutsler, J.J., Zhang, H., 2010. Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. Brain Res. 1309, 83–94. https://doi.org/10.1016/j.brainres.2009.09.120.

Iñiguez, S.D., Aubry, A., Riggs, L.M., Alipio, J.B., Zanca, R.M., Flores-Ramirez, F.J., Hernandez, M.A., Nieto, S.J., Musheyev, D., Serrano, P.A., 2016. Social defeat stress induces depression-like behavior and alters spine morphology in the hippocampus of adolescent male C57BL/6 mice. Neurobiol. Stress 5, 54–64. https://doi.org/10.1016/j.ynstr.2016.07.001.

Iñiguez, S.D., Riggs, L.M., Nieto, S.J., Dayrit, G., Zamora, N.N., Shawhan, K.L., Cruz, B., Warren, B.L., 2014. Social defeat stress induces a depression-like phenotype in adolescent male c57BL/6 mice. Stress 17, 247–255. https://doi.org/10.3109/10253890.2014.910650.

Janssen, B., Vugts, D.J., Windhorst, A.D., Mach, R.H., 2018. PET imaging of microglial activation-beyond targeting TSPO. Molecules 23. https://doi.org/10.3390/molecules23030607.

Kentner, A.C., Bilbo, S.D., Brown, A.S., Hsiao, E.Y., McAllister, A.K., Meyer, U., Pearce, B. D., Pletnikov, M.V., Yolken, R.H., Bauman, M.D., 2019. Maternal immune activation: reporting guidelines to improve the rigor, reproducibility, and transparency of the model. Neuropsychopharmacology 44, 245–258. https://doi.org/10.1038/s41386-018-0185-7.

Kim, M., Haney, J.R., Zhang, P., Hernandez, L.M., Wang, L., Perez-Cano, L., Loohuis, L. M.O., de la Torre-Ubieta, L., Gandal, M.J., 2021. Brain gene co-expression networks link complement signaling with convergent synaptic pathology in schizophrenia. Nat. Neurosci. 24, 799–809. https://doi.org/10.1038/s41593-021-00847-z.

Kim, Y.-K., Won, E., 2017. The influence of stress on neuroinflammation and alterations in brain structure and function in major depressive disorder. Behav. Brain Res. 329, 6–11. https://doi.org/10.1016/j.bbr.2017.04.020.

Koyama, R., Ikegaya, Y., 2015. Microglia in the pathogenesis of autism spectrum disorders. Neurosci. Res. 100, 1–5. https://doi.org/10.1016/j.neures.2015.06.005.

Lai, M.-C., Kassee, C., Besney, R., Bonato, S., Hull, L., Mandy, W., Szatmari, P., Ameis, S. H., 2019. Prevalence of co-occurring mental health diagnoses in the autism population: a systematic review and meta-analysis. Lancet Psychiatr. 6, 819–829. https://doi.org/10.1016/S2215-0366(19)30289-5.

Lee, J.D., Coulthard, L.G., Woodruff, T.M., 2019. Complement dysregulation in the central nervous system during development and disease. Semin. Immunol. 45, 101340 https://doi.org/10.1016/j.smim.2019.101340.

Lee, S.-Y., Oh, H.R., Kim, Y.-H., Bae, S.-H., Lee, Y., Lee, Y.-S., Lee, B.C., Cheon, G.J., Kang, K.W., Youn, H., 2022. Cerenkov luminescence imaging of interscapular brown adipose tissue using a TSPO-targeting PET probe in the UCP1 ThermoMouse. Theranostics 12, 6380–6394. https://doi.org/10.7150/thno.74828.

Li, X., Tian, X., Lv, L., Hei, G., Huang, X., Fan, X., Zhang, Jinming, Zhang, Jianjiang, Pang, L., Song, X., 2018. Microglia activation in the offspring of prenatal Poly I: C exposed rats: a PET imaging and immunohistochemistry study. Gen. Psychiatry 31, 29–36. https://doi.org/10.1136/gpsych-2018-000006.

Marques, T.R., Ashok, A.H., Pillinger, T., Veronese, M., Turkheimer, F.E., Dazzan, P., Sommer, I.E.C., Howes, O.D., 2019. Neuroinflammation in schizophrenia: meta-analysis of in vivo microglial imaging studies. Psychol. Med. 49, 2186–2196. https://doi.org/10.1017/S0033291718003057.

Mattei, D., Ivanov, A., Ferrai, C., Jordan, P., Guneykaya, D., Buonfiglioli, A., Schaafsma, W., Przanowski, P., Deuther-Conrad, W., Brust, P., Hesse, S., Patt, M., Sabri, O., Ross, T.L., Eggen, B.J.L., Boddeke, E.W.G.M., Kaminska, B., Beule, D., Pombo, A., Kettenmann, H., Wolf, S.A., 2017. Maternal immune activation results in complex microglial transcriptome signature in the adult offspring that is reversed by minocycline treatment. Transl. Psychiatry 7. https://doi.org/10.1038/tp.2017.80.

Maynard, T.M., Sikich, L., Lieberman, J.A., LaMantia, A.S., 2001. Neural development, cell-cell signaling, and the "two-hit" hypothesis of schizophrenia. Schizophr. Bull. https://doi.org/10.1093/oxfordjournals.schbul.a006887.

Meyer, U., Murray, P.J., Urwyler, A., Yee, B.K., Schedlowski, M., Feldon, J., 2008. Adult behavioral and pharmacological dysfunctions following disruption of the fetal brain balance between pro-inflammatory and IL-10-mediated anti-inflammatory signaling. Mol. Psychiatr. 13, 208–221. https://doi.org/10.1038/sj.mp.4002042.

Meyer, U., Nyffeler, M., Engler, A., Urwyler, A., Schedlowski, M., Knuesel, I., Yee, B.K., Feldon, J., 2006. The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology. J. Neurosci. 26, 4752–4762. https://doi.org/10.1523/JNEUROSCI.0099-06.2006.

Morgan, J.T., Chana, G., Pardo, C.A., Achim, C., Semendeferi, K., Buckwalter, J., Courchesne, E., Everall, I.P., 2010. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol. Psychiatr. 68, 368–376. https://doi.org/10.1016/j.biopsych.2010.05.024.

Mouri, A., Ukai, M., Uchida, M., Hasegawa, S., Taniguchi, M., Ito, T., Hida, H., Yoshimi, A., Yamada, K., Kunimoto, S., Ozaki, N., Nabeshima, T., Noda, Y., 2018. Juvenile social defeat stress exposure persistently impairs social behaviors and neurogenesis. Neuropharmacology 133, 23–37. https://doi.org/10.1016/j.neuropharm.2018.01.016.

Mueller, F.S., Scarborough, J., Schalbetter, S.M., Richetto, J., Kim, E., Couch, A., Yee, Y., Lerch, J.P., Vernon, A.C., Weber-Stadlbauer, U., Meyer, U., 2021. Behavioral, neuroanatomical, and molecular correlates of resilience and susceptibility to maternal immune activation. Mol. Psychiatr. 26, 396–410. https://doi.org/10.1038/s41380-020-00952-8.

Nutma, E., Fancy, N., Weinert, M., Marzin, M.C., Tsartsalis, S., Muirhead, R.C.J., Falk, I., de Bruin, J., Hollaus, D., Pieterman, R., Anink, J., Story, D., Chandran, S., Tang, J., Trolese, M.C., Saito, T., Saido, T.C., Wiltshire, K., Beltran-Lobo, P., Philips, A., Antel, J., Healy, L., Moore, C.S., Bendotti, C., Aronica, E., Radulescu, C.I., Barnes, S. J., Hampton, D.W., van der Valk, P., Jacobson, S., Matthews, P.M., Amor, S., Owen, D.R., 2022. Translocator protein is a marker of activated microglia in rodent models but not human neurodegenerative diseases, 2022.05.11.491453 bioRxiv. https://doi.org/10.1101/2022.05.11.491453.

Onwordi, E.C., Halff, E.F., Whitehurst, T., Mansur, A., Cotel, M.-C., Wells, L., Creeney, H., Bonsall, D., Rogdaki, M., Shatalina, E., Reis Marques, T., Rabiner, E.A., Gunn, R.N., Natesan, S., Vernon, A.C., Howes, O.D., 2020. Synaptic density marker SV2A is reduced in schizophrenia patients and unaffected by antipsychotics in rats. Nat. Commun. 11, 246. https://doi.org/10.1038/s41467-019-14122-0.

Osimo, E.F., Beck, K., Reis Marques, T., Howes, O.D., 2019. Synaptic loss in schizophrenia: a meta-analysis and systematic review of synaptic protein and mRNA measures. Mol. Psychiatr. 24, 549–561. https://doi.org/10.1038/s41380-018-0041-5.

Paolicelli, R.C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., Giustetto, M., Ferreira, T.A., Guiducci, E., Dumas, L., Ragozzino, D., Gross, C.T., 2011. Synaptic pruning by microglia is necessary for normal brain development. Science 333, 1456–1458. https://doi.org/10.1126/science.1202529.

Parise, L.F., Sial, O.K., Warren, B.L., Sattler, C.R., Duperrouzel, J.C., Parise, E.M., Bolaños-Guzmán, C.A., 2020. Nicotine treatment buffers negative behavioral consequences induced by exposure to physical and emotional stress in adolescent male mice. Psychopharmacology 237, 3125–3137. https://doi.org/10.1007/s00213-020-05598-6.

Pedersen, C.B., Mortensen, P.B., 2001. Evidence of a dose-response relationship between urbanicity during upbringing and schizophrenia risk. Arch. Gen. Psychiatr. 58, 1039–1046. https://doi.org/10.1001/archpsyc.58.11.1039.

Pendyala, G., Chou, S., Jung, Y., Coiro, P., Spartz, E., Padmashri, R., Li, M., Dunaevsky, A., 2017. Maternal immune activation causes behavioral impairments and altered cerebellar cytokine and synaptic protein expression. Neuropsychopharmacology. https://doi.org/10.1038/npp.2017.7.

Purves-Tyson, T.D., Weber-Stadlbauer, U., Richetto, J., Rothmond, D.A., Labouesse, M. A., Polesel, M., Robinson, K., Shannon Weickert, C., Meyer, U., 2019. Increased levels of midbrain immune-related transcripts in schizophrenia and in murine offspring after maternal immune activation. Mol. Psychiatr. https://doi.org/10.1038/s41380-019-0434-0.

Ransohoff, R.M., Perry, V.H., 2009. Microglial physiology: unique stimuli, specialized responses. Annu. Rev. Immunol. 27, 119–145. https://doi.org/10.1146/annurev.immunol.021908.132528.

Resende, L.S., Amaral, C.E., Soares, R.B.S., Alves, A.S., Alves-dos-Santos, L., Britto, L.R. G., Chiavegatto, S., 2016. Social stress in adolescents induces depression and brain-region-specific modulation of the transcription factor MAX. Transl. Psychiatry 6. https://doi.org/10.1038/tp.2016.202 e914–e914.

Richetto, J., Calabrese, F., Meyer, U., Riva, M.A., 2013. Prenatal versus postnatal maternal factors in the development of infection-induced working memory impairments in mice. Brain Behav. Immun. 33, 190–200. https://doi.org/10.1016/j.bbi.2013.07.006.

Sams-Dodd, F., Lipska, B.K., Weinberger, D.R., 1997. Neonatal lesions of the rat ventral hippocampus result in hyperlocomotion and deficits in social behaviour in adulthood. Psychopharmacology 132, 303–310. https://doi.org/10.1007/s002130050349.

Sano, W., Nakamura, T., Yoshiuchi, K., Kitajima, T., Tsuchiya, A., Esaki, Y., Yamamoto, Y., Iwata, N., 2012. Enhanced persistency of resting and active periods of locomotor activity in schizophrenia. PLoS One 7, e43539.

Seery, M.D., Leo, R.J., Lupien, S.P., Kondrak, C.L., Almonte, J.L., 2013. An upside to adversity?: moderate cumulative lifetime adversity is associated with resilient responses in the face of controlled stressors. Psychol. Sci. 24, 1181–1189. https://doi.org/10.1177/0956797612469210.

Selten, J.-P., Frissen, A., Lensvelt-Mulders, G., Morgan, V.A., 2010. Schizophrenia and 1957 pandemic of influenza: meta-analysis. Schizophr. Bull. 36, 219–228. https://doi.org/10.1093/schbul/sbp147.

Shimizu, T., Ishida, A., Hagiwara, M., Ueda, Y., Hattori, A., Tajiri, N., Hida, H., 2020. Social defeat stress in adolescent mice induces depressive-like behaviors with reduced oligodendrogenesis. Neuroscience 443, 218–232. https://doi.org/10.1016/j.neuroscience.2020.07.002.

Stilo, S.A., Murray, R.M., 2010. The epidemiology of schizophrenia: replacing dogma with knowledge. Dialogues Clin. Neurosci. 12 (3), 305–315. https://doi.org/10.31887/DCNS.2010.12.3/sstilo.

Tetreault, N.A., Hakeem, A.Y., Jiang, S., Williams, B.A., Allman, E., Wold, B.J., Allman, J. M., 2012. Microglia in the cerebral cortex in autism. J. Autism Dev. Disord. 42, 2569–2584. https://doi.org/10.1007/s10803-012-1513-0.

Tsuchiya, K.J., Byrne, M., Mortensen, P.B., 2003. Risk factors in relation to an emergence of bipolar disorder: a systematic review. Bipolar Disord. 5, 231–242. https://doi.org/10.1034/j.1399-5618.2003.00038.x.

Van Camp, N., Lavisse, S., Roost, P., Gubinelli, F., Hillmer, A., Boutin, H., 2021. TSPO imaging in animal models of brain diseases. Eur. J. Nucl. Med. Mol. Imag. 49, 77–109. https://doi.org/10.1007/s00259-021-05379-z.

Van Den Eynde, K., Missault, S., Fransen, E., Raeymaekers, L., Willems, R., Drinkenburg, W., Timmermans, J.P., Kumar-Singh, S., Dedeurwaerdere, S., 2014. Hypolocomotive behaviour associated with increased microglia in a prenatal immune activation model with relevance to schizophrenia. Behav. Brain Res. https://doi.org/10.1016/j.bbr.2013.10.005.

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. Schizophr. Bull. 38, 661–671. https://doi.org/10.1093/schbul/sbs050.

Vidal, J., Bie, J. de, Granneman, R.A., Wallinga, A.E., Koolhaas, J.M., Buwalda, B., 2007. Social stress during adolescence in Wistar rats induces social anxiety in adulthood without affecting brain monoaminergic content and activity. Physiol. Behav. 92, 824–830. https://doi.org/10.1016/j.physbeh.2007.06.004.

Wang, W., Liu, W., Duan, D., Bai, H., Wang, Z., Xing, Y., 2021. Chronic social defeat stress mouse model: current view on its behavioral deficits and modifications. Behav. Neurosci. 135, 326–335. https://doi.org/10.1037/bne0000418.

Wang, X., Yang, J., Zhang, H., Yu, J., Yao, Z., 2019. Oral probiotic administration during pregnancy prevents autism-related behaviors in offspring induced by maternal immune activation via anti-inflammation in mice. Autism Res. 12, 576–588. https://doi.org/10.1002/aur.2079.

Warren, B.L., Sial, O.K., Alcantara, L.F., Greenwood, M.A., Brewer, J.S., Rozofsky, J.P., Parise, E.M., Bolaños-Guzmán, C.A., 2014. Altered gene expression and spine density in nucleus accumbens of adolescent and adult male mice exposed to emotional and physical stress. Dev. Neurosci. 36, 250–260. https://doi.org/10.1159/000362875.

Warren, B.L., Vialou, V.F., Iñiguez, S.D., Alcantara, L.F., Wright, K.N., Feng, J., Kennedy, P.J., LaPlant, Q., Shen, L., Nestler, E.J., Bolaños-Guzmán, C.A., 2013. Neurobiological sequelae of witnessing stressful events in adult mice. Biol. Psychiatr. 73, 7–14. https://doi.org/10.1016/j.biopsych.2012.06.006.

Watt, M.J., Burke, A.R., Renner, K.J., Forster, G.L., 2009. Adolescent male rats exposed to social defeat exhibit altered anxiety behavior and limbic monoamines as adults. Behav. Neurosci. 123, 564–576. https://doi.org/10.1037/a0015752.

Woolway, G.E., Smart, S.E., Lynham, A.J., Lloyd, J.L., Owen, M.J., Jones, I.R., Walters, J. T.R., Legge, S.E., 2022. Schizophrenia polygenic risk and experiences of childhood adversity: a systematic review and meta-analysis. Schizophr. Bull. 48, 967–980. https://doi.org/10.1093/schbul/sbac049.

Yizhar, O., Fenno, L.E., Prigge, M., Schneider, F., Davidson, T.J., O'Shea, D.J., Sohal, V. S., Goshen, I., Finkelstein, J., Paz, J.T., Stehfest, K., Fudim, R., Ramakrishnan, C., Huguenard, J.R., Hegemann, P., Deisseroth, K., 2011. Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature 477, 171–178. https://doi.org/10.1038/nature10360.

Yoon, S., Kim, Y.-K., 2020. In: Kim, Y.-K. (Ed.), The Role of the Oxytocin System in Anxiety Disorders BT - Anxiety Disorders: Rethinking and Understanding Recent Discoveries. Springer Singapore, Singapore, pp. 103–120. https://doi.org/10.1007/978-981-32-9705-0_7.

Zeng, L.-L., Shen, H., Liu, L., Wang, L., Li, B., Fang, P., Zhou, Z., Li, Y., Hu, D., 2012. Identifying major depression using whole-brain functional connectivity: a multivariate pattern analysis. Brain 135, 1498–1507. https://doi.org/10.1093/brain/aws059.

Zhao, J., Bao, A.-M., Qi, X.-R., Kamphuis, W., Luchetti, S., Lou, J.-S., Swaab, D.F., 2012. Gene expression of GABA and glutamate pathway markers in the prefrontal cortex of non-suicidal elderly depressed patients. J. Affect. Disord. 138, 494–502. https://doi.org/10.1016/j.jad.2012.01.013.

Zhu, F., Zheng, Y., Liu, Y., Zhang, X., Zhao, J., 2014. Minocycline alleviates behavioral deficits and inhibits microglial activation in the offspring of pregnant mice after administration of polyriboinosinic–polyribocytidilic acid. Psychiatr. Res. 219, 680–686. https://doi.org/10.1016/j.psychres.2014.06.046.