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*Published in:*
Behavioural Brain Research

*DOI:*
10.1016/j.bbr.2023.114566



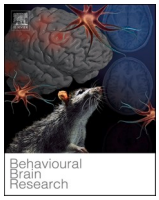
*Publication date:*
2023

Behavioural Brain Research 452 (2023) 114566

Contents lists available at ScienceDirect

Behavioural Brain Research

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



Research report

# Maternal infection during pregnancy aggravates the behavioral response to an immune challenge during adolescence in female rats

Cyprien G.J. Guerrin [a], Erik F.J. de Vries [a], Kavya Prasad [a], Daniel A. Vazquez-Matias [a], Lesley E. Manusiwa [a], Lara Barazzuol [b,c], Janine Doorduin [a,*]

[a] Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Hanzeplein 1, Groningen 9713 GZ, the Netherlands
[b] Department of Radiation Oncology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, Groningen 9713 GZ, the Netherlands
[c] Department of Biomedical Sciences of Cells and Systems, University of Groningen, University Medical Center Groningen, Hanzeplein 1, Groningen 9713 GZ, the Netherlands

ARTICLE INFO

*Keywords:*
Psychopathologies
Poly I:C
Lipopolysaccharides
Microglia
LPS challenge
Maternal immune activation

ABSTRACT

Prenatal and early postnatal infection have been associated with changes in microglial activity and the development of psychiatric disorders. Here, we investigated the effect of prenatal immune activation and postnatal immune challenge, alone and combined, on behavior and microglial cell density in female Wistar rats. Pregnant rats were injected with poly I:C to induce a maternal immune activation (MIA). Their female offspring were subsequently exposed to a lipopolysaccharide (LPS) immune challenge during adolescence. Anhedonia, social behavior, anxiety, locomotion, and working memory were measured with the sucrose preference, social interaction, open field, elevated-plus maze, and Y-maze test, respectively. Microglia cell density was quantified by counting the number of Iba-1 positive cells in the brain cortex. Female MIA offspring were more susceptible to the LPS immune challenge during adolescence than control offspring as demonstrated by a more pronounced reduction in sucrose preference and body weight on the days following the LPS immune challenge. Furthermore, only the rats exposed to both MIA and LPS showed long-lasting changes in social behavior and locomotion. Conversely, the combination MIA and LPS prevented the anxiety induced by MIA alone during adulthood. MIA, LPS, or their combination did not change microglial cell density in the parietal and frontal cortex of adult rats. The results of our study suggest that the maternal immune activation during pregnancy aggravates the response to an immune challenge during adolescence in female rats.

## 1. Introduction

Immune activation during pregnancy or in early life are environmental risk factors associated with psychiatric disorders such as schizophrenia, autism, attention deficit hyperactivity disorder (ADHD) and depression [1–4]. Especially an increased risk to develop schizophrenia is linked to maternal exposure to infections agents. Influenza (3–7-fold increase), herpes, respiratory infections in the second semester (3-fold increase), toxoplasmosis (1–2-fold increase) and rubella (20 % of prenatally rubella-exposed subjects diagnosed with schizophrenia as adults/ 10–20 fold risk increase) have been associated with increased risk of schizophrenia [5,6]. Maternal infection was also shown to increase the risk of offspring childhood infections, which together render the child more vulnerable to psychosis development [7]. In addition, hospitalization for infection during childhood was associated with a two-fold increased risk of adult psychosis [7–9]). However, given their relatively frequent occurrence, pre- or postnatal immune activation alone is usually not sufficient to induce a pathological phenotype and seems to have a modest effect in large populations [10–12]. For example, despite about 40 % of the population being infected during influenza pandemics, only a marginal increase (relative risk ratios of 1–2.5) of the global incidence of schizophrenia was observed [12,13]. Therefore, it has been proposed that prenatal maternal infection may prime the individual to become more responsive to the pathological effects of a second postnatal immune challenge such as infection or sepsis during adolescence, a period of high susceptibility as many neurodevelopmental processes are still taking place [14–17].

Many of these neurodevelopmental processes are regulated by the

* Corresponding author.
*E-mail address:* j.doorduin@umcg.nl (J. Doorduin).

https://doi.org/10.1016/j.bbr.2023.114566
Received 2 March 2023; Received in revised form 23 May 2023; Accepted 3 July 2023
Available online 5 July 2023

immune system, which has been hypothesized to be altered by the combination of prenatal and adolescent infection [18]. This altered activity of the immune system may be characterized by a change in the number and activity of microglial cells, which are not only involved in the immune defense of the brain, but also in many neurodevelopmental processes [19,20]. Increased neuroinflammatory markers like brain levels of pro-inflammatory cytokines and reactive microglia are often observed in patients with schizophrenia [21–23].

Rodent models of maternal immune activation (MIA), by administering pregnant animals with an immune stimulant, are known to induce behavioral and neuropathological alterations in the offspring consistent with schizophrenia [24]. Preclinical data on microglial changes in MIA offspring is contradictory, as some studies reported no change in microglial density and morphology in adolescent and adult MIA offspring [25–29], while others reported an increase in microglia density [27,30–33]. Studies investigating the effect of a neonatal or adolescent immune challenge are inconsistent as some have shown changes in anxiety, social and exploratory behavior that remained until adulthood, while other studies did not observe such changes [34–36]. An adolescent immune challenge was also shown to increase pro-inflammatory and decrease anti-inflammatory responses in the brain 24 h after the adolescence immune challenge [37]. This increased immune response was significantly higher in male offspring prenatally exposed to MIA [37]. However, whether similar susceptibility can also be observed in female MIA offspring remains unclear and requires further investigation, considering the gender differences noted in human psychiatric disorders associated with maternal infection [24,38,39]. Gender differences include women having a higher lifetime prevalence of mood and anxiety disorder than men and a later onset of schizophrenia psychoses for example [40–44]. Recent research have also demonstrated sex-specific microglial signatures in both patients with psychiatric disorders and preclinical models, that may contribute to the symptoms' differences [35]. Furthermore, the possible long-term effect of pre- and postnatal infection on microglial activity and behavior remains unclear.

In this study, we tested whether maternal immune activation (MIA), an adolescent immune challenge, or their combination can affect behavior and microglial activity in adult female rats. To model the effects of MIA, we administered the viral mimic polyinosinic: polycytidylic acid (poly I:C) known to trigger a wide range of behavioral and neuronal abnormalities related to schizophrenia [24,45]. To model the effects of an immune challenge during adolescence we administered lipopolysaccharide (LPS), a major component of the outer membrane of gram-negative bacteria, a factor shown to induce an acute inflammatory response in humans and rodents [46,47]. Symptoms often observed in schizophrenia were measured. Abnormalities in social behavior, anxiety, anhedonia have been repeatedly associated with dysfunction of the immune system notably in cerebral cortex [48–51]. Therefore, microglial cells density was examined by quantification of cells immunoreactive for the ionized calcium-binding adaptor molecule 1 (Iba1) in the parietal and frontal cortices, brain regions notably involved in the studied behavior [52–54].

## 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 of the Netherlands. Eleven 11-weeks old male and female Wistar rats (strain HsdCpb:WU) were purchased from Envigo (Horst, The Netherlands) and left for acclimatization for at least seven days before breeding. Breeding consisted of placing a male rat in the cage of a female rat and leave them overnight for a total duration of 36 h (from early morning to late afternoon the next day). When the males were removed was considered as gestational day 1. Pregnancy was identified by vaginal plugs, weight gain and physical appearances. Pregnant rats were housed individually until weaning on postnatal day (PND) 21. The offspring were randomly housed in groups of 2 or 3 rats in humidity-controlled (55–60 %) and thermo-regulated (21 ± 2 °C) rooms, with a 12:12-h light:dark cycle (lights on at 7 a.m.). Food and water were available ad libitum. Only the female offspring were used in this study.

### 2.2. Experimental design

MIA was induced by injecting pregnant rats with poly I:C, a viral mimic, on gestational day 15 (GD15). Control rats were injected with saline. Female offspring were randomized and intraperitoneally injected with saline or 1 mg/kg of bodyweight of LPS on PND36. Female offspring were randomly divided into four groups: (1) control (Control, n = 13 from 5 different litters), (2) rats from mothers injected with poly I:C (MIA, n = 12 from 5 different litters), (3) rats injected with LPS during adolescence (LPS, n = 12 from 6 different litters), and (4) rats from mothers injected with poly I:C and injected with LPS during adolescence (MIA+LPS, n = 12 from 6 different litters). All the offspring rats were exposed to all the behavioral tests that were performed on day 37–44 and 97–100 (Fig. 1A). More specifically, the elevated-plus maze (EPM) was conducted on day 41 and 97, the open field (OFT) test was conducted on day 42 and 98, the social interaction test was conducted following the OFT on day 42 and 98 and the Y-maze was conducted on day 44 and 100. The behavioral tests were conducted during the light phase of the rats. Brains were collected on day 105 to measure microglial density.

### 2.3. Maternal immune activation

On GD15, pregnant dams were put under anesthesia with 5 % isoflurane in oxygen and intravenously injected in the tail vein with 4 mg/kg poly-I:C in saline (MIA, n = 6) or saline (control, n = 6). The timing and dosage are commonly reported for this rat model [55,56]. Poly-I:C potassium salt (Sigma-Aldrich) was dissolved in 0.9 % NaCl solution to yield a final concentration of 2 mg/mL. All poly I:C solutions were freshly prepared on the day of administration. After the poly-I:C administration, animals were allowed to wake up, returned to their home cages and checked for possible sickness behavior. A guideline checklist for the methodological details of the MIA model can be found in the supplemental materials [57]. To reduce the litter effect, one to three female offspring from the same litter were used per group.

### 2.4. LPS injection during adolescence

On PND36, half of rats were intraperitoneally injected with 1 mg/kg bodyweight of E. coli LPS (Sigma Aldrich, L2630) dissolved in 1 mL of saline. The other half was intraperitoneally injected with a saline solution. The 1 mg/kg bodyweight was selected after a pilot study during which three different doses were tested (0.5, 1 and 2 mg/kg). As similar behavior in the OFT, EPM and SPT was observed in rats injected with 1 and 2 mg/kg of LPS, we selected the lower dose. To induce an immune challenge during adolescence, rats were injected on day 36 as conducted by a previous study [37]. Rats were checked for possible sickness behavior (reduced motility in the cage, immobility, reduced water and food intake, piloerection) and were weighted daily for 5 consecutive days.

### 2.5. Sucrose preference test

Sucrose preference tests (SPT) were performed on PND37 (the first night after the LPS immune challenge), 41, and 97 to measure anhedonia. The sucrose preference test was conducted on PND37 and 41 to determine the immediate and possible short-lasting effect of LPS on anhedonia (sucrose preference). Before the test, the animals underwent 4 consecutive days of SPT training consisting of placing a bottle with 1 %

sucrose in tap water in the cage for 1 h. After the 4 days of SPT training and 2 overnight SPT training sessions, the SPT was performed on PND37, 41, and 97. The overnight SPT training sessions and SPT consisted of overnight exposure to two bottles, one filled with drinking water, and one filled with a 1 % sucrose solution dissolved in drinking water. The sucrose preference was measured as the outcome parameter and was calculated according to the formula: sucrose preference (%) = [Sucrose intake (mL)/ (Sucrose intake (mL)+ Water intake (mL))] *100 %.

### 2.6. Elevated-plus maze

The EPM test was performed on PND41 and 97 to measure anxiety and locomotion. The EPM arena is placed at an elevation of 62 cm and is composed of four 50 cm long arms (2 opposite open arms and two opposite arms enclosed by high walls) and a center area allowing the rats to travel from one arm to another. After 30 min of acclimatization in the experimental room, the rat was placed in the center of the EPM arena facing a closed arm and allowed to freely explore for 5 min, a duration often selected for the EPM [58,59] Time spent in open and close arms and in the center, the number of entries in the open arms and the total distance travelled were measured.

### 2.7. Open field test

The OFT was performed on PND42 and 98 to measure anxiety and locomotion. Rats were placed in the experimental room at least 30 min before the test to acclimatize. The OFT consisted of placing the rat in the periphery of a 100 cm diameter circular area for 5 min, similarly to what was conducted by previous studies [29,58,60]. The center was defined as a circle with a 70 cm diameter. The time spent in the center, number of entries in the center and total distance travelled were measured.

### 2.8. Social interaction test

SIT was performed following the open field test on PND42 and 98 to measure social behavior. To stimulate and motivate social behavior, the rats were socially isolated for 2 h prior to testing [61,62]. The experimental rat and an unfamiliar rat were placed within a 50 * 50 cm square arena for 5 min. Rats were considered unfamiliar if they had never previously encountered the experimental rats. Unfamiliar rats were matched for with the experimental rats for age, sex and bodyweight. Eight different unfamiliar rats were used. The experimental rat was considered as interacting when it was displaying social behavior toward the unfamiliar rat. Grooming, sniffing, licking, following closely and playing behavior (pinching, pouncing, fighting) were considered as social behavior and not differentiated during the analysis.

### 2.9. Y-maze

The Y-maze test was performed on PND44 and 100 to measure working memory and locomotion. The y-maze arena is a Y shape arena composed of three 50 cm arms intersected at an angle of 120 degrees. After at least 30 min of acclimatization to the experimental room, the rat was placed in the center of a Y-maze for a total duration of 8 min [58]. Spontaneous alternation was defined as entering three different arms consecutively. The alternation index was calculated using the following formula: alternation index = [(number of spontaneous alternations) /(total number of arm entries - 2) * 100 Alternation index and total locomotion were measured.

### 2.10. Analysis of behavior

The EPM, OFT and Y-maze were video recorded and analyzed offline using Ethovision XT14 (Noldus Information Technology, Wageningen, The Netherlands). The center point of the animal was used to automatically track the rat's behavior. The social interaction test was video recorded and manually scored by two independent examiners that were blinded to the experimental groups. In the SPT, 2 control, 1 MIA and 1 LPS rats on PND37, 1 control on PND41 and 1 control on PND97 were excluded from the analysis due to leaking or obstructed bottles found in the following morning. In the EPM, 1 adolescent control, 1 adolescent MIA, 4 adolescent LPS, 2 adolescent MIA+LPS and 3 MIA+LPS rats were excluded because the fell off the EPM during the data collection. No data is missing from the SIT and OFT.

### 2.11. Brain collection and immunohistochemistry

On PND105, rats were put under deep anesthesia and the heart was perfused with PBS. The right hemisphere was isolated and fixed in 4 % paraformaldehyde (PFA) at room temperature for 48 h. Subsequently, the brains were dehydrated in a 25 % sucrose solution at 4 °C, embedded in optimal cutting temperature (OCT) compound and stored at −80 °C.

For immunohistochemistry staining, 15 μm sagittal brain sections were prepared by cryosection. After washing three times with PBS, antigen retrieval was performed by pressure cooking for 10 min in 10 mM sodium citrate, pH 6.0. The sections were subsequently washed and incubated in phosphate-buffered saline (PBS) with 0.3 % hydrogen peroxide for 30 min to block endogenous peroxidases. After washing again, the sections were blocked for 30 min with 2 % normal donkey serum (Jackson Immuno Research, 017–000-121) in PBS with 1 % Triton X-100 ($PBS^+$) and 2 % bovine serum albumin (BSA). The sections were then incubated with the primary rabbit-α-ionized calcium-binding adapter molecule 1 (Iba1) antibody (1:2000; Wako, 01–19741) with $PBS^+$ and 1 % BSA overnight at 4 °C. The following day, the sections were washed and incubated with the biotinylated secondary donkey-α-rabbit IgG antibody (1:400; Jackson Immuno Research, 711–065-152) for 2 h. After washing with PBS, the sections were incubated with Avidin/Biotinylated enzyme Complex ABC solution (VECTASTAIN® ABC Kit, Vector Laboratories, PK-6100) for 30 min. The sections were washed and stained using 0.04 % 3,3′-diaminobenzidine and 0.03 % hydrogen peroxide for 10 min and subsequently dehydrated using a sequence of increasing ethanol concentrations (50–100 %). After being air-dried, the slides were mounted with coverslips using DePex (Serva) and stored at room temperature before imaging using light microscopy.

### 2.12. Microglial density and spatial distribution analysis

The density of microglia in the parietal and frontal cortices was determined by counting all Iba1 positive cells in 6–8 region of interest of known dimensions (0.28 $mm^2$) per cortical region and animal using ImageJ software (http://rsb.info.nih.gov/ij/). The spatial distribution of microglia in the cortex and the distance of the microglia to their nearest neighbor, that is, the average Euclidian distances between the nearest cells, were determined using the NND plugin for ImageJ. Five MIA, LPS, MIA+LPS rats, and six controls rats were used.

### 2.13. Statistical analysis

Statistical analyses of body weight, and behavior were performed using SPSS (IBM SPSS Statistics, Version 22.0). A generalized estimating equation (GEE) analysis was performed for longitudinal data, using 'MIA', 'LPS' and 'age' as factors, as this analysis can account for missing data and was adjusted for multiple comparisons using the "least significant difference". Wald Chi-square (W) and degrees of freedom (df) are presented for the GEE analysis. A one-way ANOVA using GraphPad 8 software was performed to assess differences between groups in Iba1 staining, as it was assessed at only a single time point and no data was missing. The data are presented as mean ± standard deviation (SD).

## 3. Results

### 3.1. Previous exposure to MIA induced a longer reduction in bodyweight following an LPS challenge during adolescence

The bodyweight of all groups increased with age (W=9636, df=14, $p < 0.001$, Fig. 1B). We observed an interaction between LPS and age (W=287, df=1, $p < 0.0001$). On PND37, the day following the LPS injection, pairwise comparison analyses (W=3E+13, df=45, $p < 0.0001$) revealed that LPS and MIA+LPS rats had a lower bodyweight than control (−11 %, $p < 0.001$; −6 %, $p = 0.002$) and MIA rats (−15 %, $p < 0.001$; −10 %, $p = 0.001$). While this reduction was not observed in LPS rats anymore on PND38, MIA+LPS rats still had a lower bodyweight than control and MIA rats on PND38 (−14 %, $p < 0.001$; −9 %, $p = 0.011$), PND39 (−20 %, $p < 0.006$; −14 %, $p = 0.001$), and PND40 (−15 %, $p < 0.028$; −6 %, $p = 0.003$).

### 3.2. Previous exposure to MIA aggravates anhedonia following an LPS challenge during adolescence

The sucrose preference test was used to assess anhedonia (Fig. 1C). We observed a main effect of age (W=22, df=2, $p < 0.001$) and a LPS*age interaction (W=19, df=2, $p < 0.001$). On PND37, pairwise analysis (W=50, df=11, $p < 0.001$) revealed that rats exposed to the

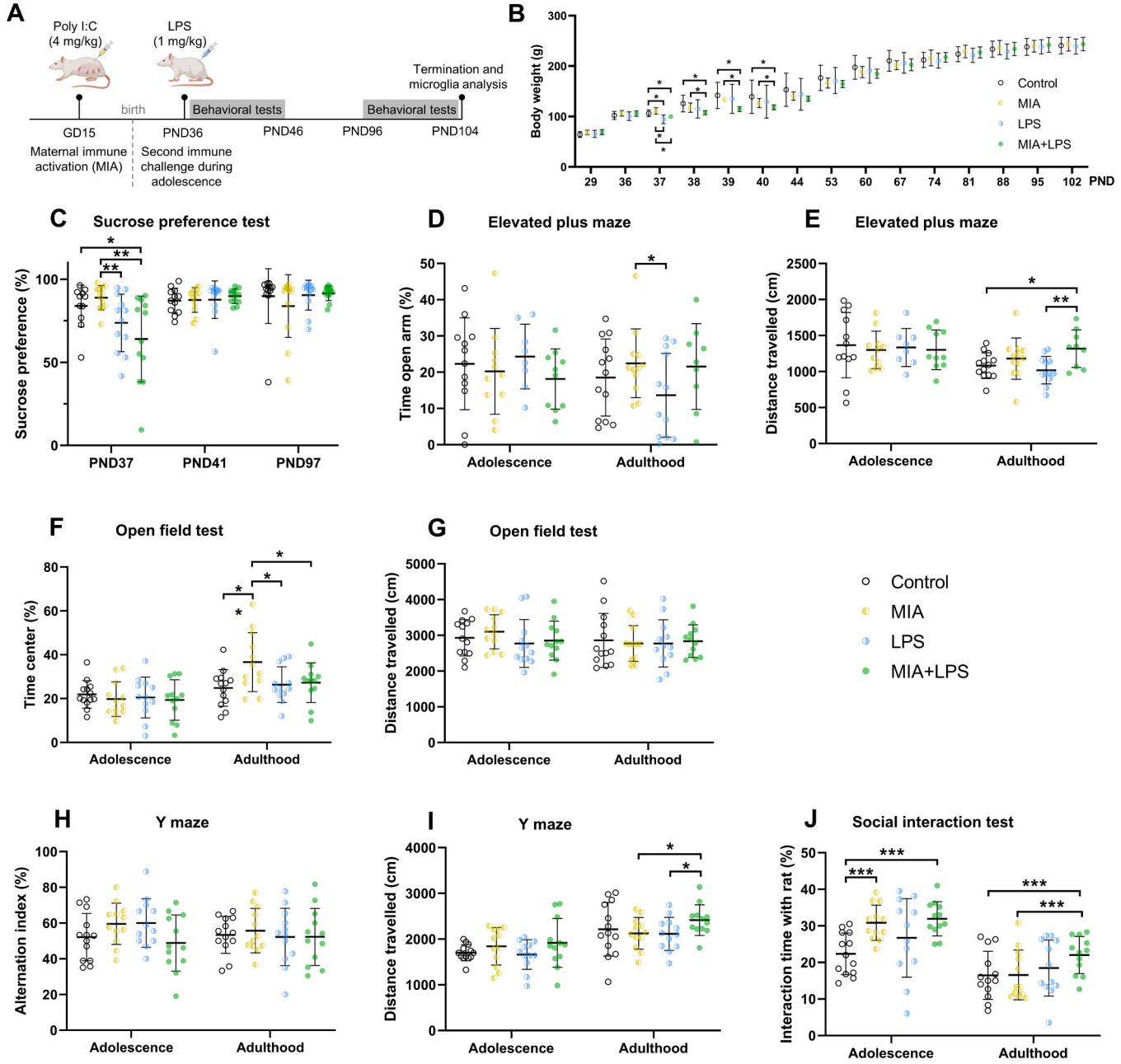



**Fig. 1.** Study design, bodyweight, anhedonia, social behavior, anxiety, locomotion and working memory changes. **A.** Study design. Pregnant dams were intravenously injected with either saline or poly I:C on gestational day (GD) 15. Behavioral experiments conducted during adolescence (PND36–46) and adulthood (PND96–104). Brains were collected for iba1 staining on PND105. **B.** Bodyweight. **C.** Anhedonia in the sucrose preference test. **D.** Social behavior in the social interaction test. **E.G.** Anxiety in the EPM and the OFT. **I.** Working memory in the Y-maze. **F.H.J.** Locomotion. N = 11–13 rat per group. Data is presented as mean ±SD. Statistically significant differences between groups were perfected using GEE analysis and are indicated by asterisks: *$p < 0.05$, * *$p < 0.01$, * **$p < 0.001$. Significant differences between time points are not shown.

combination of MIA and LPS had a lower sucrose preference than control (−24 %, p = 0.013) and MIA rats (−28 %, p < 0.001). LPS rats had a lower sucrose preference than MIA rats (−18 %, p = 0.003), but not control rats. No differences between groups were observed on PND41 and PND97.

### 3.3. The combination of MIA and LPS increased locomotion during adulthood in the EPM

The EPM was used to assess anxiety (Fig. 1D) and locomotion (Fig. 1E). We observed no difference in the EPM during adolescence.

We observed an interaction between MIA and age in the percentage of time spent in the open arm (W=7, df=1, p = 0.007). In adulthood, pairwise analysis (W=11, df=7 p = 0.118) revealed that MIA rats spent longer time in the open arms than LPS rats (+57 %, p = 0.033).

We observed a main effect of age on the distance travelled in the EPM (W=11, df=1, p < 0.001) and an interaction between MIA and age (W=5, df=1, p = 0.021). In adulthood, pairwise analysis (W=31, df=7, p < 0.001) revealed that the rats exposed to the combination of MIA and LPS traveled significantly more than control (+22 %, p = 0.013) and LPS rats (+29 %, p = 0.002).

We observed no significant difference in time spent in the center, time spent in the closed arms, or the number of entries in the open arms (Supplementary Fig. 1A–C).

### 3.4. LPS exposure during adolescence prevented the MIA-induced reduction in anxiety during adulthood in the OFT

The percentage of time spent in the center of the arena and the total distance travelled in the OFT were used to assess anxiety (Fig. 1F) and locomotion (Fig. 1G), respectively.

We observed a main effect of age (W=27, df=1, p < 0.001) and an interaction between MIA and age (W=6, dl=1, p = 0.013) on the time spent in the center. In adulthood, pairwise analysis (W=30, df=7, p < 0.001) revealed that MIA rats spent more time in the center of the open field than control (+48 %, p = 0.006), LPS (+42 %, p = 0.018) and MIA+LPS rats (+37 %, p = 0.036). We observed no differences in distance travelled in the OFT between groups.

### 3.5. The combination of MIA and LPS reduced working memory in adolescence and increased locomotion during adulthood in the Y-maze

Spontaneous alternations and distance travelled in the Y-maze were measured to assess working memory (Fig. 1H) and locomotion (Fig. 1I), respectively.

We observed neither an interaction nor a main effect of MIA, LPS or age on the alternation index.

We observed a main effect of age on the distance travelled (W=41, df=1, p < 0.001). In adulthood, pairwise analysis (W=93, df=7, p < 0.0001) revealed that MIA+LPS rats travelled significantly more than MIA (+14 %, p = 0.030) and LPS rats (+15 %, p = 0.027).

### 3.6. MIA increased social behavior during adolescence and adulthood

To test social behavior, we quantified the preference of a rat for interacting with an unfamiliar rat (Fig. 1J). We observed a main effect of MIA (W=9, df=1, p = 0.003), LPS (W=5, df=1, p = 0.028), and age (W=71, df=1, p < 0.001) and an interaction between MIA and age (W=5, df=1, p = 0.027). In adolescence, pairwise comparisons (W=116, df=7, p < 0.0001), revealed that MIA (+41 %, p < 0.001) and MIA+LPS rats (+45 %, p < 0.001) spent more time interacting with the unfamiliar rat than control rats. This effect persisted only for MIA+LPS rats. In adulthood, the rats exposed to the combination of MIA and LPS had a higher interaction time than control (+38 %, p = 0.013) and MIA rats (+29 %, p = 0.020).

### 3.7. MIA and LPS did not affect microglia density in the parietal and frontal cortex during adulthood

To determine the effect of MIA and LPS on microglia, microglial cell density was determined in the parietal and frontal cortices by counting the number of Iba1-positive cells (Fig. 2A). In adulthood, we observed no differences between groups in the density of microglial cells (Fig. 2B. C.) or the nearest neighbor distance between microglial (Fig. 2D.E.) in the parietal and frontal cortices.

## 4. Discussion

The present findings indicate that prenatal maternal immune activation increased the behavioral susceptibility of the female offspring to an immune challenge during adolescence (Fig. 3.). More specifically, female MIA offspring had a more pronounced reduction in sucrose preference the day after an immune challenge during adolescence than healthy rats. Furthermore, MIA offspring exposed to the adolescent immune challenge showed long-lasting changes in social behavior and locomotion that were not observed if rats were exposed to MIA or LPS alone. Conversely, the MIA alone reduced anxiety in adulthood, while the combination of MIA and LPS prevented such reduction. Prenatal immune activation, adolescent immune challenge, or their combination did not change microglial cell density in the parietal and frontal cortex of adult rats.

MIA exposure is a well-validated rodent model that induces behavior that is similar to the positive, negative and cognitive symptoms observed in schizophrenia and other psychiatric disorders [24]. Male MIA rat offspring from mothers exposed to 4 mg/kg iv or 10 mg/kg ip of poly I:C on GD15 (similar to our study) were observed to display a reduction [63–65] or no change in social behavior or an increase in aggressive behavior while non-aggressive behavior remained normal [66,67] in the social interaction test (rats freely interacting). In contrast, in our study, MIA alone increased the duration of social interaction during adolescence but not adulthood, which might be due to MIA rats seeking for social comfort to reduce stress. In line with our study, another study observed that male rat MIA (5 mg/kg poly I:C, GD 15) offspring had an increase in social behavior [65,68] characterized by an increase in anogenital sniffing. They suggested that an increase in anogenital sniffing may be an indication of impaired social recognition resulting from difficulties to identify and to accommodate to a novel rat. Perhaps a similar deficit could explain the increased social behavior observed in our study. Future studies should investigate in more depth the different types of social playing behavior during adolescence (sniffing, pouncing, and pinning); as well as social behavior in adulthood (anogenital sniffing, sniffing, aggressive behavior, grooming, climbing, following, duration, and number of episodes of social interplay) as it could provide a better insight in the social behavior that may be altered by MIA. Future studies should also consider measuring pure social behavior in addition to the commonly measured social preference in the 3-chamber test.

We also found that adult female offspring exposed to MIA spent more time in the center of the open field arena, as compared to control rats suggesting a reduction in anxiety levels. However, in line with other studies [29,69,70], MIA rat (4 mg/kg poly I:C, GD14–15) offspring did not show differences in any parameters in the EPM, which is considered a more robust test used to assess anxiety. Taken together, the results from the OFT and EPM may suggest mild anxiety induced by MIA. Another possible explanation is that MIA offspring took longer to habituate and accommodate to the arena and thus spent longer time exploring its center. Finally, we observed that female rats exposed to MIA had no significant changes in anhedonia, locomotion, and working memory in the SPT, OFT, EPM, and Y-maze, respectively. Previous studies with rats exposed to MIA (4 mg/kg poly I:C, GD14) have shown contradicting results, as some of them support our findings such as no change in sucrose preference, working memory, and locomotion [28, 69], while other studies exposing mice to MIA (5 mg/kg, GD12–15) have

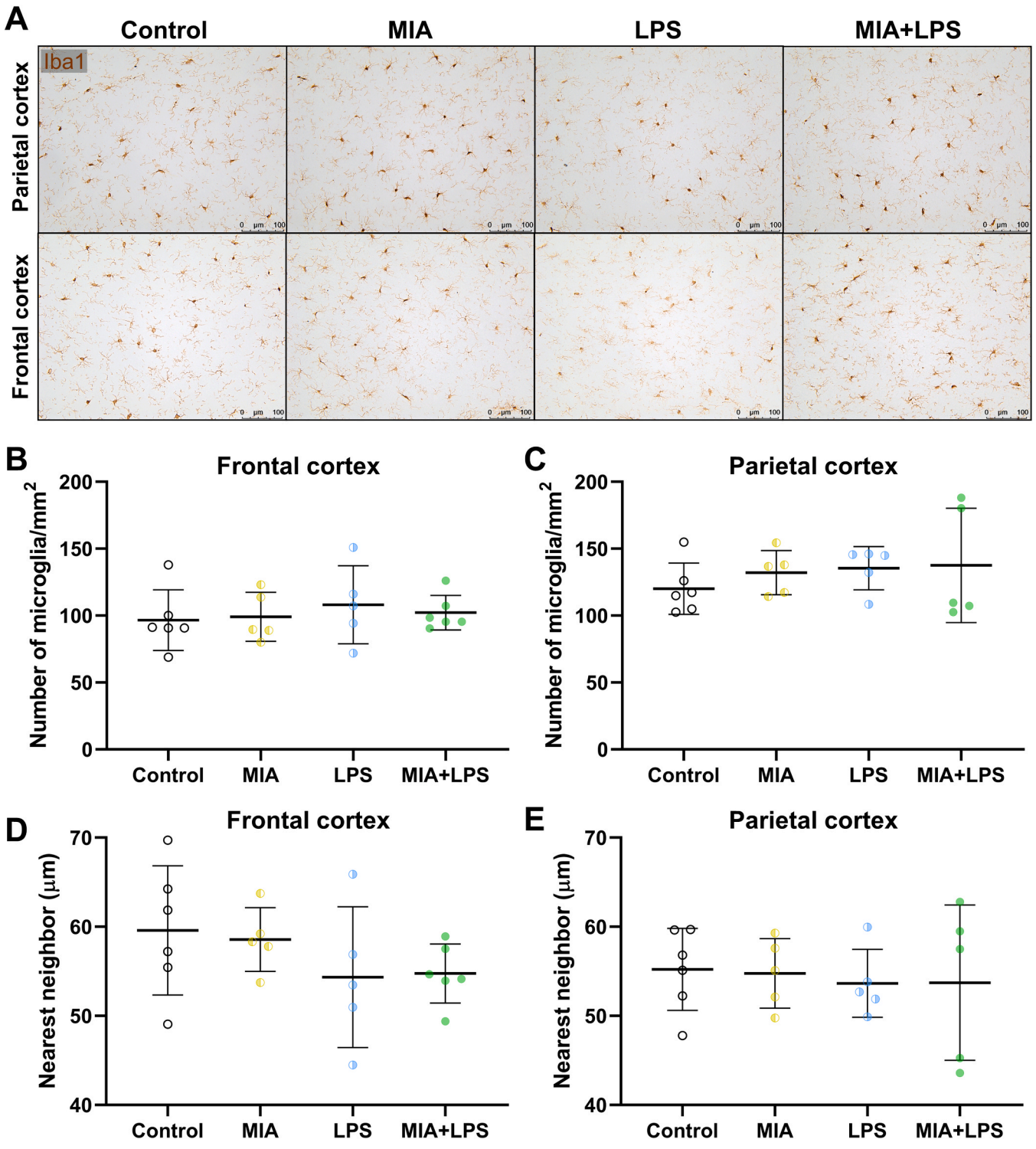



**Fig. 2.** Density of microglia in adulthood. Representative iba1 staining of microglia in the parietal and frontal cortices of Control, MIA, LPS, and MIA+LPS groups (**A**). Number of Iba1-positive cells/mm$^2$ in the frontal (**B**), and parietal (**C**) cortices. Nearest neighbor distance analysis in the frontal (**D.**) and parietal cortices (**E.**). n = 5–6 rats per group. Graphs represent mean ± SD.

reported significant alterations in these behavioral outcomes [71–73]. Differences in the timing of the maternal immune activation and in species may explain these differences, as they have been shown to critically determine the patterns of behavioral abnormalities displayed in the offspring at adult age, as early maternal immune activation was shown to induce anxiety, while late MIA did not [74,75].

Injection of LPS is a well-validated model to challenge the immune system. In the present study, LPS injected during adolescence (day 36) induced immediate but not long-lasting sickness behavior characterized by a reduced body weight and sucrose preference in SPT. This is consistent with findings in animal studies demonstrating sickness behavior following LPS injection (0.5–2 mg/kg / day 35–60) [37,46] and clinical data indicating similar behavior in response to an LPS injection or an infection [47]. The observed LPS effects on behavior were more pronounced when rats were previously exposed to MIA suggesting an additive effect of LPS and MIA. For example, sucrose preference

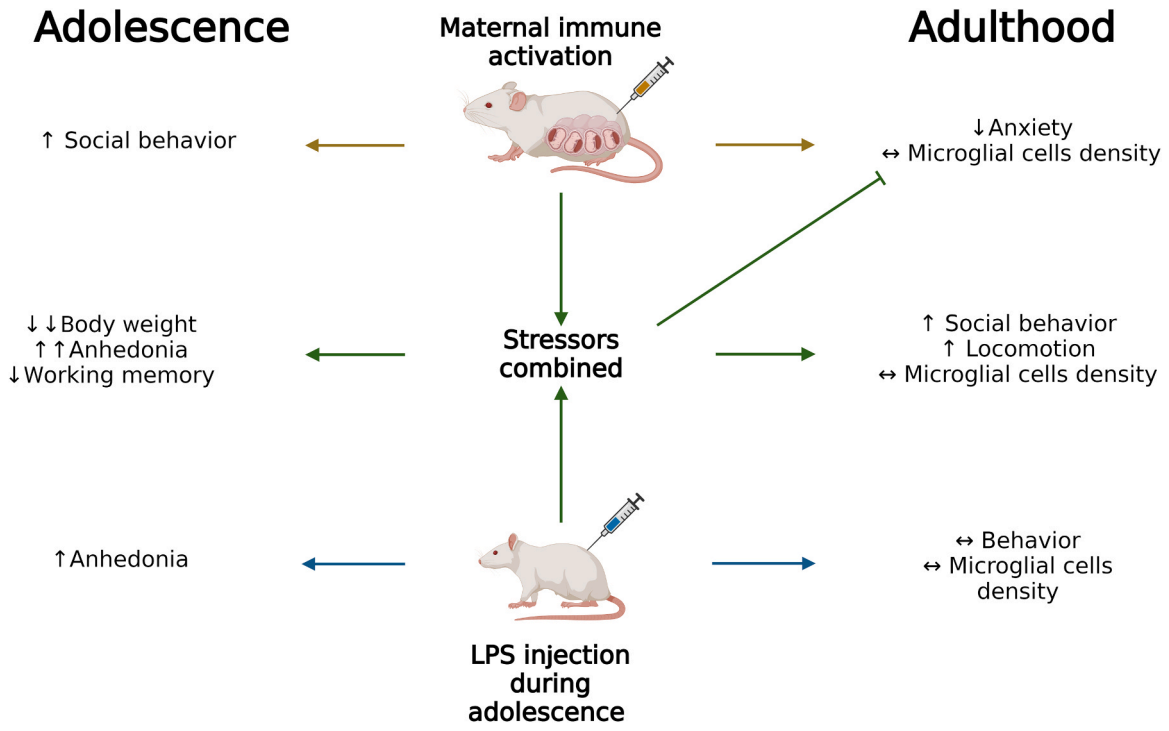



**Fig. 3.** Summary figure of the effect of MIA, LPS during adolescence and their combination on behavior and microglia. Arrows up and down represent significant differences compared to control animals or animals exposed to one of the two stressors on the same day. ↔ = no significant changes, ↑ = increase, ↑↑ = additive increase, ↓ = decrease, ↓↓= additive decrease. Created with BioRender.com.

dropped significantly more in the female rats exposed to both LPS and MIA. Furthermore, only the combination of MIA and LPS induced a reduction in body weight, which remained up to 5 days after the LPS challenge. In addition, female MIA offspring exposed to LPS displayed an increase in social behavior during adolescence that remained until adulthood. This effect was not observed in rats exposed to MIA or adolescent LPS administration alone. LPS injection (0.05 mg/kg) was shown to promote social behavior in adult MIA (20 mg/kg ip, GD 12.5) male offspring by increasing the proinflammatory cytokine IL-17a [76]. Perhaps the cumulative production of IL-17a induced by MIA and LPS could explain the long-lasting increase in social behavior observed in our study. We also observed that the combination of MIA and LPS during adolescence induced hyperlocomotion in adulthood in the EPM and Y-maze but not OFT. This could be related to the design of the arenas used to measure the paradigms. The open field arena confronts the animals to a wide-open space, while the EPM and Y-maze feature high walls which could increase the feeling of security. This design difference could result in the increased locomotion found in the EPM and Y-maze, but not in the OFT. Hyperlocomotion is often associated with alterations in the mesolimbic dopaminergic pathway [77], a pathway involved in schizophrenia for example. Alterations in this dopaminergic pathway may explain the behavioral changes observed in our study as this system is also involved in regulating social behavior [78,79]. Our results are in line with clinical studies showing that hyperactivity symptoms are common in patients with autism [80] and can be observed in patients with schizophrenia [81]. Deviant activity and density of microglia, the main immune cells of the brain, has been associated with several psychiatric disorders including schizophrenia. In our study, we did not find any differences in the number of $Iba1^+$ cells in the parietal and frontal cortices, regions involved in the symptomatology of many psychiatric disorders, of adult female rats exposed to MIA, adolescent immune challenge, or their combination, as compared to control rats. The preclinical data on microglial changes in MIA offspring is contradictory. While some studies reported no change in microglial density and morphology in adult MIA (4–5 mg/kg poly I:C, GD15) offspring [25, 27–29,73,82], others reported an increase in density of microglia and morphological changes in the hippocampus, and neocortex [66], or even a decrease in reactivity in some brain regions [27,32,66]. The timing of the poly I:C immune activation is also important as MIA on GD9 or 12 induced significant changes in microglia morphology and density while it did not have a significant effect on GD15 [27,30,31,83]. Clinical studies give similar conflicting results as some studies showed low to moderate levels of neuroinflammation in schizophrenia patients, whereas other studies did not detect any change in microglial density and activity [84–86]. A possible explanation might be that a change in microglia activity is mostly present in certain neurodevelopmental stages such as during early development and following the immune challenge during adolescence. Supporting this hypothesis, male MIA (5 mg/kg of poly I:C iv, GD15) offspring exposed to an LPS (2 mg/kg, day 35) immune challenge during adolescence were shown to have a synergistic increase in brain pro-inflammatory cytokines and a reduction in anti-inflammatory cytokines [37]. Another study observed that MIA (1 mg/kg of poly I:C iv, GD9) combined with adolescent stressors induced a transient increase in microglial activity that resulted in behavioral changes in adulthood [87]. Perhaps MIA in female offspring induced similar transient changes in the immune system, which could help to explain the short- and long-term impairment in behavior, such as the aggravated reduction in sucrose preference in the SPT following the LPS challenge and alterations in social behavior and locomotion in adulthood that were observed only if the rats were exposed to the combination of MIA and LPS. This possible deviant activity of the immune system following the adolescent LPS exposure, a critical developmental period, possibly characterized by a subtle change in the number and activation status of microglia, may alter neurodevelopmental processes such as synaptic pruning. Synaptic pruning was shown to be dysregulated in neurodevelopmental disorders such as schizophrenia and autism [19,20,88] [89,90]. These immune changes may induce attenuated or excessive synaptic pruning that would result in lower or higher synaptic connectivity, as observed in schizophrenia and autism, respectively [19,20]. Positron emission tomography (PET)

imaging and post-mortem studies observed 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 in patients with schizophrenia [91–93]. On the other hand, a higher spine density [94], disrupted synapse excitatory versus inhibitory balance [95] and hyperactivity in frontal brain regions [96] was observed in patients with autism spectrum disorders. Future studies should investigate further the possible link between prenatal and adolescent infection, the immune system, synaptic pruning and synaptic density. Our study includes some limitations, such as the absence of direct measurement of inflammatory cytokines, such as interleukin 1β and interleukin 10, in brain and plasma. Furthermore, our longitudinal study design did not allow the assessment of possible changes in microglia and the immune system shortly after the adolescent LPS immune challenge. As previously mentioned, MIA (5 mg/kg of poly I:C iv, GD15) was shown to increase the susceptibility of the male offspring to an adolescent immune challenge (2 mg/kg of LPS) characterized by a synergistic increase in pro-inflammatory and decrease in anti-inflammatory responses in the brain 24 h after the adolescent immune challenge [37]. We suggest that similar changes would be induced in our rat model. Finally, only females were included in this study, thus preventing to determine a possible sex difference.

## 5. Conclusion

Our results indicate that prenatal maternal immune activation increased susceptibility of the female offspring to behavioral impairment after an adolescent immune challenge. This increased susceptibility was characterized by a pronounced reduction in body weight and anhedonia behavior during the days following the adolescent immune challenge, as well as changes in social behavior and locomotion in adulthood. These alterations were not observed if only one stressor was applied. These symptoms mimic the symptoms associated with neurodevelopmental disorders. For example, anhedonia, altered social behavior, and hyperactivity are often observed in patients with autism. The MIA-induced enhanced susceptibility to an immune challenge during adolescence was not accompanied by an increase in microglial density in adulthood. Future studies are needed to investigate the immune response to the combination of MIA and immune challenge during adolescence in more detail to better understand the behavioral changes observed in our model.

## Formatting of funding sources

This research did not receive any specific grant 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, the members of the Baron's groups and Jurgen Sijbesma for the experimental support.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2023.114566.

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