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Ontogeny and plasticity of resilience and susceptibility in a mouse model of maternal immune activation

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ABSTRACT

Maternal immune activation (MIA) during pregnancy results in variable neurodevelopmental and behavioral outcomes in both humans and animal models. In a mouse model of MIA using prenatal poly(I:C) administration, we recently identified subgroups of MIA-exposed offspring with distinct behavioral and transcriptional profiles even under genetic homogeneity. Here, we used the same model to explore whether the expression of resilient and susceptible phenotypes after MIA represents stable traits or whether they exhibit plasticity throughout adolescent maturation. Conducting longitudinal testing in a first cohort, we revealed that MIA offspring can be stratified into subgroups with distinct behavioral profiles at juvenile age. This early divergence was sex-dependent and predictive of different behavioral outcomes at adult age. In a second cohort, we examined the effects of repeated social intervention during peri-adolescence on brain and behavioral trajectories. In male MIA offspring displaying juvenile deficits in sociability and hyperactivity, the intervention did not alleviate adult deficits in sociability or temporal order memory but prevented the adult emergence of prepulse inhibition impairments. Conversely, in female MIA offspring with juvenile social deficits, the intervention improved adult deficits in sociability and temporal order memory, but it failed to normalize adult impairments in prepulse inhibition. These sex-specific behavioral outcomes were paralleled by subgroup-specific changes in oxytocinergic and dopaminergic markers in cortical and subcortical brain regions. Together, our findings indicate that MIA-exposed offspring can be stratified into distinct subgroups early in life, with subsequent risk and resilience trajectories varying by sex. Moreover, our data identify a window of plasticity during which targeted interventions can modulate abnormal maturational trajectories, ultimately mitigating the long-term effects of MIA in a sex-dependent manner.

1. Introduction

Maternal immune activation (MIA), whether triggered by infectious or non-infectious factors during pregnancy, is a transdiagnostic environmental risk factor for various psychiatric and neurodevelopmental disorders (Brown and Meyer, 2018; Careaga et al., 2017; Gumusoglu and Stevens, 2019; Meyer, 2019; Vasistha and Sawa, 2025). Multiple pathophysiological processes connect MIA to these disoders, such as inflammation and oxidative stress affecting both the mother and fetus, activation of maternal stress pathways, transient nutrient deficiencies, and impaired placental function. (Bilbo et al., 2018; Meyer, 2019, 2014; Puglisi et al., 2025; Weber-Stadlbauer, 2017). These processes can disrupt the development of somatic cells and alter neurodevelopmental trajectories, increasing the likelihood of cognitive and behavioral deficits in later stages of life. Moreover, epigenetic changes have been identified as a crucial mechanism through which MIA produces lasting effects on brain function, potentially influencing gene expression and behavior across successive generations (Basil et al., 2018; Hayes et al., 2022; Labouesse et al., 2015; Richetto et al., 2017; Richetto and Meyer, 2021; Weber-Stadlbauer, 2017; Weber-Stadlbauer et al., 2021, Weber-Stadlbauer et al., 2017).

Although accumulating evidence highlights its possible health consequences, the outcomes of MIA on the offspring are variable. Some children exposed to MIA *in utero* may develop central nervous system (CNS) abnormalities, while others do not (Fajardo-Martinez et al., 2024; Hornig et al., 2018; Jaswa et al., 2024; Jones et al., 2017; Mahic et al.,

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2017). Hence, a significant level of resilience to MIA exists, influencing how well offspring are protected from neurodevelopmental consequences (Meyer, 2019). This heterogeneity is also reflected in the variable effects of MIA observed in animal models (Meyer, 2023; Weber-Stadlbauer and Meyer, 2019). Similar to many other model systems (Jaswa et al., 2024; Kafkafi et al., 2018; Voelkl and Würbel, 2024), the specificity of MIA-induced effects in laboratory animals is influenced by several factors, including dosing and specificity of the immune-activating agents (Bao et al., 2022; Meyer et al., 2005; Missig et al., 2020; Mueller et al., 2018; Yotova et al., 2024, 2024), timing of MIA (Guma et al., 2022; Meehan et al., 2017; Meyer et al., 2006, 2008b; Richetto et al., 2017), genetic background (Abazyan et al., 2010; Lipina et al., 2013; Schwartzer et al., 2013; Vuillermot et al., 2012), age and sex of the offspring (Crum et al., 2017; Garay et al., 2013; Giovanoli et al., 2015; Gogos et al., 2020; Missig et al., 2020; Richetto et al., 2014; Vernon et al., 2015; Woods et al., 2023), rearing environment (Connors et al., 2014; Mueller et al., 2018; Zhao et al., 2021b), and intrauterine positioning of fetuses (Schaer et al., 2024). All these factors introduce intended or unintended variability in animal models of MIA, presenting both opportunities and challenges for preclinical MIA research (Kentner et al., 2019; Meyer, 2023; Weber-Stadlbauer and Meyer, 2019).

Even under strictly controlled experimental conditions, substantial variability exists in animal models of MIA. For instance, using rodent models of MIA induced by prenatal administration of the viral mimetic poly(I:C) (*polyriboinosinic-polyribocytidylic acid*), recent studies identified distinct subgroups of MIA-exposed offspring exhibiting divergent pathological profiles (Herrero et al., 2023; Lorusso et al., 2022; Mueller et al., 2021). In our own studies, we found that susceptible MIA offspring displayed overt deficits in multiple behavioral functions and brain networks, whereas resilient MIA offspring did not (Mueller et al., 2021). Furthermore, we observed distinct transcriptional patterns in both cortical and subcortical brain regions, along with variations in innate inflammatory cytokine production, between susceptible and resilient subgroups (Herrero et al., 2023; Mueller et al., 2021). Notably, the dissociation into susceptible and resilient subgroups emerged even under genetically uniform conditions, identical MIA exposure, and strictly regulated laboratory settings (Herrero et al., 2023; Mueller et al., 2021), suggesting that factors beyond genetic background and initial immune activation contribute to the heterogeneity of outcomes (Schaer et al., 2024).

Thus far, however, MIA-exposed offspring in animal models have been classified as susceptible or resilient based solely on biobehavioral and biochemical measurements taken in adulthood. Therefore, it remains to be determined whether similar subgroups exist at earlier ages or emerge progressively during adolescent maturation. It also remains elusive whether the phenotypic expression of resilience and susceptibility represents stable traits or whether they exhibit plasticity throughout maturation. Identifying behavioral markers that differentiate resilient from susceptible offspring could provide valuable insights into the developmental trajectories underlying these phenotypic differences. Moreover, understanding the temporal dynamics of resilience and susceptibility in the context of MIA may identify potential windows of plasticity during which targeted interventions could modulate abnormal maturational trajectories, ultimately mitigating the long-term effects of MIA.

The present study aimed to address these issues using a mouse model in which MIA was induced by maternal administration of poly(I:C) during pregnancy. In a first cohort of animals, we stratified the behavioral profiles of control and MIA offspring at the juvenile age and followed them up to assess the same behavioral readouts in adulthood. This longitudinal investigation served to ascertain whether subgroups of MIA offspring exhibit differing behavioral profiles at the juvenile age and whether these subgroup-specific differences remain stable from juvenile to adult age. In a second cohort of control and MIA offspring, we evaluated whether targeted behavioral interventions could attenuate the emergence of adult behavioral phenotypes. Based on findings from the first cohort, the behavioral intervention was implemented as repeated social engagement across adolescence. This second cohort was also used for immunohistochemical analyses of oxytocinergic and dopaminergic markers in cortical and subcortical brain areas. The central oxytocin and dopamine systems were chosen due to their critical involvement in social behavior, locomotor and exploratory activity (Beninger, 1983; Brennan and Arnsten, 2008; Chen et al., 2007; Sakamoto et al., 2019), and sensorimotor gating (Lacroix et al., 2000; Vuillermot et al., 2010; Zhang et al., 2000, Zhang et al., 2015). All investigations were conducted in male and female offspring to identify possible sex-dependent effects.

2. Materials and methods

2.1. Animals

C57BL/6N mice were utilized for all experiments in the study. Male and female breeding pairs, aged 12 weeks, were sourced from Charles River Laboratories (Sulzfeld, Germany). Upon arrival, animals were housed in individually ventilated cages (IVCs; Allentown Inc., Bussy-Saint-Georges, France), as described before (Mueller et al., 2018). The cages were kept in a specific-pathogen-free (SPF) holding room with controlled temperature (21 ± 3 °C) and humidity (50 ± 10 %), maintained under a reversed light–dark cycle (lights off from 9:00 AM to 9:00 PM). Throughout the study, all animals had unrestricted access to standard rodent chow (Kliba 3336, Kaiseraugst, Switzerland) and water. All experimental procedures were reviewed and approved by the Cantonal Veterinarian's Office in Zurich, Switzerland.

2.2. Breeding and maternal manipulations

Timed pregnancies were established through in-house breeding, initiated two weeks after the animals had acclimated to the facility. For this purpose, male and female breeders underwent a timed-mating protocol, following previously described protocols (Mueller et al., 2018). Successful mating was confirmed by the detection of a vaginal plug, after which the females were single housed for the duration of pregnancy. The day the plug was observed was designated as gestational day (GD) 0. A female displaying a vaginal plug on GD 0 and gaining at least 3 g by GD 12 was classified as successfully pregnant (Mueller et al., 2019).

On GD 12, pregnant mice were randomly assigned to a single injection of low molecular weight (LMW) poly(I:C) obtained from InvivoGen (Toulouse, France; cat.#: tlrl-picw) or treatment with pyrogen-free 0.9 % NaCl (B. Braun, Melsungen, Switzerland) vehicle solution. The same lot of poly(I:C) (lot #PIW-41–05) was used throughout the study. We previously ascertained the quality, molecular composition and immunopotency of this poly(I:C) product (Mueller et al., 2019; Tillmann et al., 2024). Based on our previous dose response studies in C57BL/6N mice (Mueller et al., 2019; Tillmann et al., 2024), poly(I:C) was administered intraperitoneally (i.p.) at 10 mg/kg, using an injection volume of 10 ml/kg. Control (CON) dams received the equivalent volume of vehicle solution only. Immediately after poly(I:C) or vehicle administration, the dams were placed back to their home cages and left undisturbed until 5 days after birth.

Two cohorts of dams were used in this study, both of which were generated via identical on-site breeding and exposed to the same treatments. In cohort 1, the number of dams subjected to CON and MIA treatment was $n = 5$ and $n = 7$, respectively; in cohort 2, the number of dams subjected to CON and MIA treatment was $n = 8$ and $n = 10$, respectively. Additional methodological details regarding the maternal manipulations are summarized in the reporting guideline checklist for the MIA model (Kentner et al., 2019), as provided in **Supplementary Table S1**.

2.3. Allocation and testing of offspring

Offspring born to CON and MIA dams were weaned on postnatal day (PND) 21. Littermates of the same sex were housed separately and maintained in groups of 2 to 4 per cage unless specified otherwise.

For cohort 1, we employed a whole-litter testing approach, including all male and female offspring from each MIA and CON litter (Mueller et al., 2021). This cohort underwent longitudinal behavioral testing during the juvenile (PND 28–35) and early adult (PND 70 onwards) stages (Fig. 1**A**). The behavioral assessment included tests for exploratory and locomotor activity (open field test), sociability (social interaction test), and sensorimotor gating (prepulse inhibition [PPI] of the acoustic startle reflex).

In cohort 2, male and female offspring from each MIA and CON litter first underwent a social interaction test on PND 21 to obtain juvenile sociability and locomotor activity scores. These scores were used to stratify juvenile offspring into distinct subgroups (Fig. 1**B**). The animals were then housed in pairs according to their cluster membership for subsequent social interventions and longitudinal follow-up investigations (Fig. 1**B**). The rationale for examining the effects of social intervention on subgroup-specific behavioral trajectories stemmed from findings in the first cohort of animals, which suggested that deficits in juvenile sociability may play a crucial role in the emergence of subgroup-specific behavioral alterations in adulthood. The social intervention involved weekly dyadic interactions between MIA offspring with low juvenile sociability scores and CON offspring with high juvenile sociability scores ("social partners"; Fig. 1**B**). During each intervention, one MIA offspring and one social partner of the same sex were

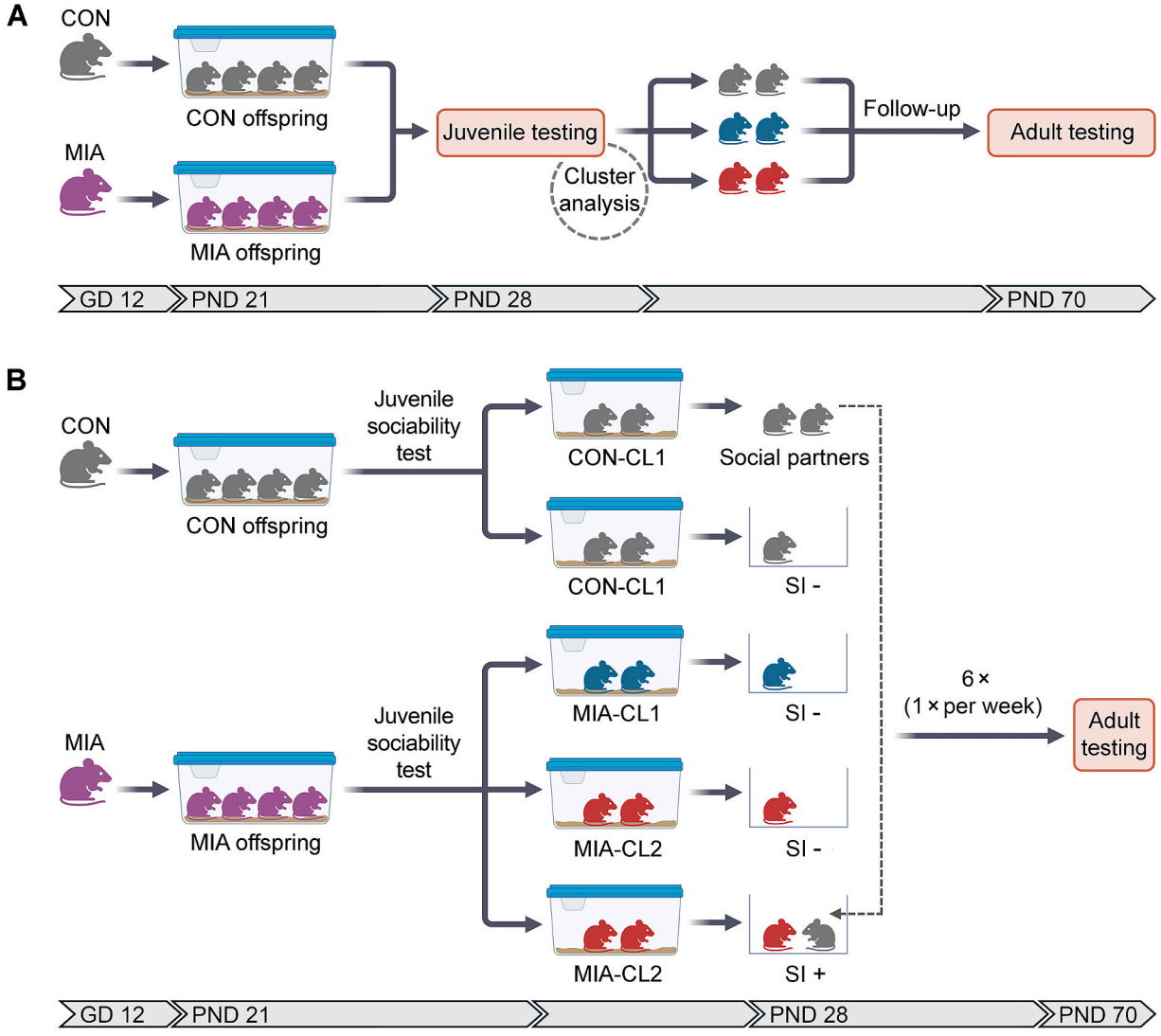

Fig. 1. Schematic illustration of the study design. **(A)** Longitudinal testing of cohort 1. Pregnant dams were exposed to control (CON) treatment or maternal immune activation (MIA) on gestation day (GD) 12. The resulting offspring first underwent behavioral testing during juvenile age (postnatal day [PND] 28–35). Unsupervised two-step cluster analysis was then performed to identify subgroups with behavioral profiles at juvenile age. Following the stratification of juvenile offspring, the subgroups were followed up into adulthood to assess the same behavioral parameters again (PND 70 onwards). Animals highlighted in red and blue color represent distinct subgroups identified through cluster analysis. **(B)** Experimental design used to study the impact of social intervention during peri-adolescence on subgroup-specific behaviors and neurochemical markers in adulthood. CON and MIA offspring were subjected to a juvenile sociability test on postnatal PND 21 to identify subgroups based on juvenile sociability and locomotor activity scores (see *Suppl. Fig. S3*). Animals highlighted in blue (MIA-CL1) and red (MIA-CL2) color represent distinct subgroups identified through cluster analysis. Starting from PND 28, subsets of MIA offspring with low juvenile sociability with (male MIA-CL2) or without (female MIA-CL2) concomitant locomotor hyperactivity were subjected to repeated social intervention (SI +) involving free dyadic social interaction with unfamiliar social partners. The latter were CON offspring with high juvenile sociability (CON-CL1). MIA-CL2 offspring receiving SI + were compared to MIA-CL2 offspring that did not receive social intervention (SI-) during peri-adolescence. CON-CL1 and MIA-CL1 offspring that did not receive social intervention (SI −) served as negative control groups. The social intervention took place once per week for a total of six weeks, after which adult behavioral parameters were assessed.

placed in a standard open field (see below), allowing them to interact freely for 5 min. The intervention began on PND 28 and was repeated weekly throughout adolescence until early adulthood. Hence, a total of 6 social interventions were applied for each designated MIA offspring (Fig. 1**B**), whereby new social partners were used for every intervention. Each intervention session was recorded by a digital camera mounted directly above the open field. To obtain measures for dyadic interactions (Jabarin et al., 2022), an experimenter blinded to treatment conditions analyzed video recordings for nose-to-nose contacts and anogenital sniffing during each social intervention. As a negative control intervention, stratified CON or MIA offspring were placed in the open field for the same duration without a social partner. Hence, the sham intervention was also conducted weekly throughout adolescence until early adulthood (Fig. 1**B**). Upon reaching early adulthood (PND 70 onwards), all animals in cohort 2 underwent behavioral and cognitive testing. Akin to cohort 1, all animals in cohort 2 were tested for sociability and sensorimotor gating in the social interaction test and PPI test, respectively. In addition, all animals from cohort 2 were subjected to a temporal order memory test for objects. This short-term memory test measures the animals' capacity to discriminate the relative recency of stimuli and is highly dependent on the prefrontal cortex (Barker et al., 2007; Schalbetter et al., 2022; von Arx et al., 2023). The rationale of using this test in cohort 2 was based on previous findings suggesting that social engagement during peri-adolescence critically shapes PFC-dependent cognitive functions in adulthood (Allen and Morishita, 2024; Hinton et al., 2019; Park et al., 2021). One week after completion of testing, the offspring in cohort 2 were euthanized for brain tissue collection and subsequent immunohistochemical analyses (see below).

Only MIA offspring that showed behavioral deficits at baseline received a social intervention, while those that did not show such deficits did not receive the intervention. This decision was based on our hypothesis that the intervention would specifically improve behavioral deficits present at baseline. In contrast, we did not anticipate measurable changes in MIA offspring without baseline deficits, due to potential ceiling effects.

2.4. Behavioral testing

Behavioral testing included the open field test, social interaction test, PPI test of the acoustic startle reflex, and temporal order memory test. Detailed methodological descriptions for each test are provided in the **Supplementary Information**. At each testing age (Fig. 1), a three-day rest period was imposed between individual tests.

2.5. Immunohistochemistry and microscopy

One week after the completion of behavioral and cognitive testing, the animals in cohort 2 were euthanized for brain sample collection to perform immunohistochemical analyses of oxytocinergic and dopaminergic markers in cortical and subcortical regions. Detailed methodological descriptions of the perfusion, brain collection, and immunohistochemical procedures are provided in the **Supplementary Information**. The density and intensity of oxytocin (OXT)-immunoreactive cells were analyzed in the paraventricular nucleus (PVN) of the hypothalamus (Bregma: −0.7 to −1.1 mm), the main brain region containing OXT-producing neurons (Grinevich and Neumann, 2021). Oxytocin receptor (OXTR) immunoreactivity was assessed in key limbic brain areas receiving oxytocinergic inputs from PVN OXT + cells (Grinevich and Neumann, 2021), including the medial prefrontal cortex (mPFC; Bregma: +2.0 to + 1.6 mm), basolateral amygdala (BLA; Bregma: −1.0 to −1.8 mm), central amygdala (CeA; Bregma: −1.0 to −1.8 mm), and CA1, CA3 and dentate gyrus (DG) regions of the hippocampus (Bregma: −1.6 to −2.2 mm). Tyrosine hydroxylase (TH) immunoreactivity was quantified in brain areas receiving major dopaminergic inputs, including mPFC (Bregma: +2.0 to + 1.6 mm), nucleus accumbens (NAc; Bregma: +1.7 to + 0.9 mm), and caudate putamen (CPu; Bregma: +1.2 to + 0.4 mm). The reason for selecting TH as dopaminergic marker of interest was because it is the rate-limiting enzyme of dopamine (and noradrenalin) synthesis *in vivo* (Bacopoulos and Bhatnagar, 1977) and found to be altered by MIA (Aguilar-Valles et al., 2020; Meyer et al., 2008a; Vuillermot et al., 2012, 2010). Detailed methodologies for microscopy and image analysis are provided in the **Supplementary Information**.

2.6. Statistical analysis

All statistical analyses were performed using SPSS Statistics (version 29.0, IBM, Armonk, NY, USA) and Prism (version 10.0; GraphPad Software, La Jolla, California), with statistical significance set at $p < 0.05$. To identify subgroups of MIA offspring with differing behavioral profiles in cohort 1, the main behavioral readouts (total distance and center zone distance moved in the open field, social preference index obtained in the social interaction test, and mean % PPI of the acoustic startle reflex) of CON and MIA offspring were analyzed by unsupervised two-step cluster analysis (Herrero et al., 2023; Mueller et al., 2021). The behavioral readouts from each individual CON and MIA offspring were fed into the cluster analysis without predetermining the number of clusters, thereby avoiding bias in terms of identifying cluster numbers (Mueller et al., 2021; Purves-Tyson et al., 2021). The Bayesian information criterion (BIC) was used to estimate the maximum number of clusters, and the log-likelihood method was used as the distance measure (Herrero et al., 2023; Mueller et al., 2021). Cluster rations were analyzed using Chi-square ($\chi 2$) tests. Following stratification of CON and MIA offspring, one-way analysis of variance (ANOVA) and Tukey's post-hoc test for multiple comparisons were used to compare the main behavioral scores between subgroups. Unsupervised two-step cluster analysis was also used to stratify juvenile CON and MIA offspring in cohort 2, whereby the social preference index and total distance moved during the social interaction test were used as input variables. Behavioral data from adult offspring in cohort 2 were analyzed by one-way ANOVA, followed by Tukey's post-hoc test for multiple comparisons. One-way ANOVA and Tukey's post-hoc test for multiple comparisons were also used for the analysis of all immunohistochemical data. All analyses were separately conducted for male and female animals.

3. Results

3.1. Identification and follow-up of male subgroups with distinctive behavioral profiles at juvenile age

In juvenile male offspring, an unsupervised two-step cluster analysis revealed two distinct subgroups (CL1 and CL2) with robust cluster separation, indicated by a silhouette coefficient exceeding 0.65. Out of the total, 25 males were categorized into CL1, while 13 were placed in CL2. The majority of male CON offspring (88.2.%, 15 out of 17) belonged to CL1 (Fig. 2**A**). In contrast, only 47.6 % (10 of 21) of the male offspring exposed to MIA fell into CL1, with the remaining 52.4 % (11 of 21) classified as CL2 (Fig. 2**A**). The distribution ratio between CL1 and CL2 significantly differed between juvenile male CON and MIA groups ($\chi^2 = 6.89$, $z = 2.62$, $p < 0.01$).

As shown in Fig. 2**B**, the distance moved in the center zone during the open field test had the highest predictor importance for cluster separation, followed by total distance moved in the open field and the social preference index. Prepulse inhibition (PPI) of the acoustic startle reflex showed minimal predictor importance for distinguishing clusters in juvenile male offspring (Fig. 2**B**). The subsequent comparison of CON-CL1, MIA-CL1 and MIA-CL2 subgroups confirmed that only MIA-CL2, but not MIA-CL1 offspring, exhibited significantly increased levels of center zone activity (ANOVA: $F_{(2,33)} = 53.69$, $p < 0.001$; CON-CL1 or MIA-CL1 versus MIA-CL2: $p < 0.001$) and total activity (ANOVA: $F_{(2,33)} = 9.17$, $p < 0.001$; CON-CL1 versus MIA-CL2: $p < 0.01$, MIA-CL1 versus MIA-CL2: $p < 0.001$) as compared to CON-CL1 offspring (Fig. 2**C**). Interestingly,

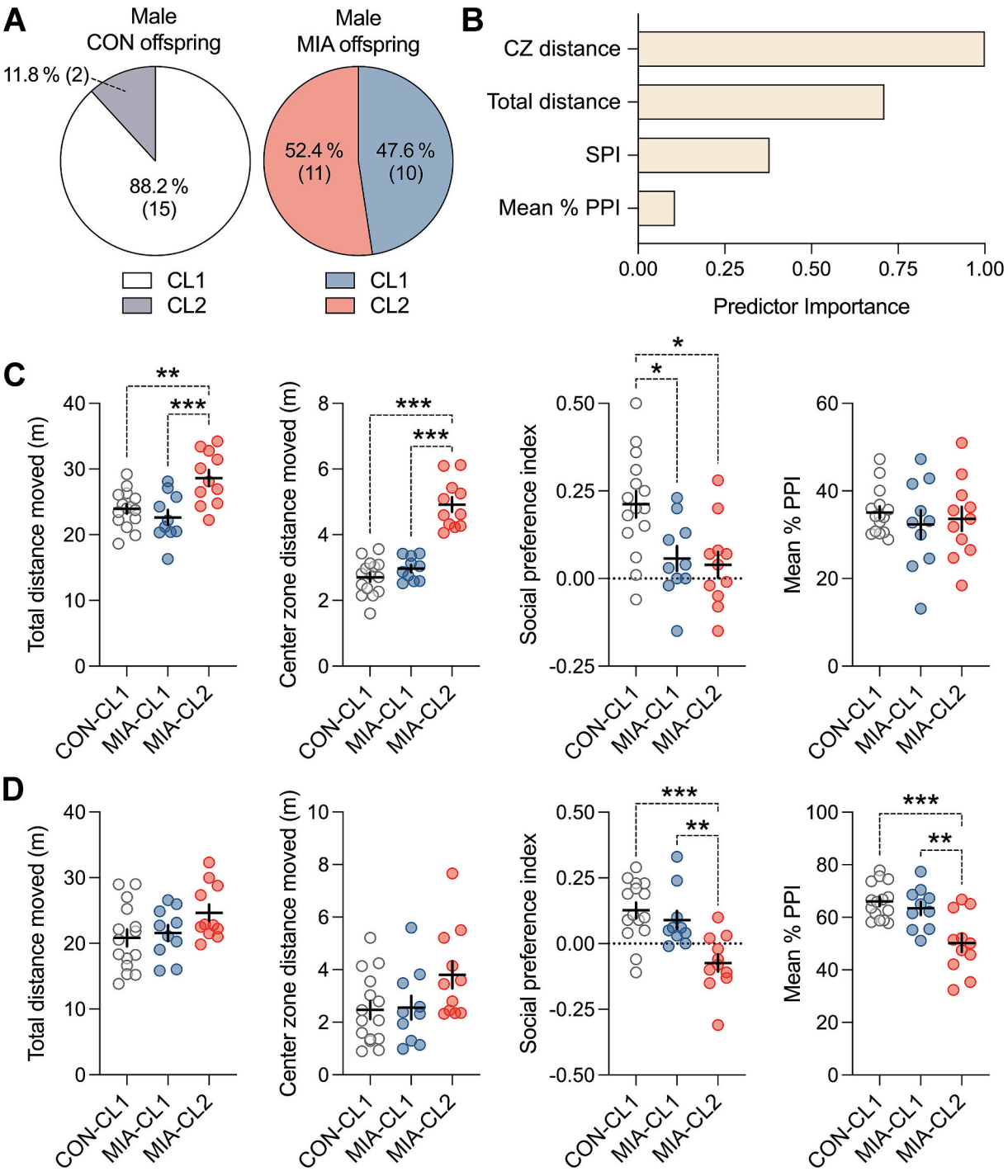

Fig. 2. Stratification of male offspring into subgroups with differing juvenile and adult behavioral profiles. A two-step cluster analysis incorporating juvenile behaviors (total distance and center zone [CZ] distance moved in the open field test; social preference index [SPI] in the social interaction test; mean percent prepulse inhibition [mean % PPI] in the PPI test) was performed to identify subgroups with differing behavioral profiles at juvenile age. The same subgroups were then retested in adulthood. **(A)** Distribution of juvenile male control (CON; $n = 17$) offspring and offspring exposed to maternal immune activation (MIA; $n = 21$) across the two clusters (CL1 and CL2) identified by two-step cluster analysis. The pie chart shows the cluster distribution (in percentages, %) for CON and MIA offspring, with numbers in brackets representing the number of male offspring in each cluster. **(B)** Relative predictor importance for cluster separation in juvenile male offspring. **(C)** Juvenile behaviors for male subgroups of CON and MIA offspring as identified by two-step cluster analysis. The plots show total distance moved (m), CZ distance moved (m), social preference index, and mean % PPI. **(D)** Adult behaviors for the same subgroups of offspring that were stratified based on juvenile behaviors. All scatter plots show individual mice with overlaid group means ± s.e.m; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, based on Tukey's post-hoc test after ANOVA.

both MIA-CL1 and MIA-CL2 subgroups showed a significant reduction in the social preference index compared to CON-CL1 offspring (ANOVA: $F_{(2,33)} = 6.93$, $p < 0.01$; CON-CL1 versus MIA-CL1 or MIA-CL2: $p < 0.05$; Fig. 2**C**). Mean % PPI levels did not differ between subgroups of juvenile male offspring (Fig. 2**C**). Together, these findings show that subgroups of MIA-exposed offspring with differing behavioral traits are identifiable already at juvenile age.

After the stratification of juvenile males, the subgroups were

followed up into adulthood to assess the same behavioral parameters again. At adult age, these subgroups no longer differed in terms of center zone and total distance moved in the open field test (Fig. 2**D**). However, adult male offspring in the MIA-CL2 subgroup continued to display a decrease in the social preference index compared to adult CON-CL1 offspring, whereas adult male offspring in the MIA-CL1 subgroup did not (ANOVA: $F_{(2,33)} = 10.82$, $p < 0.001$; CON-CL1 versus MIA-CL2: $p < 0.001$; MIA-CL1 versus MIA-CL2: $p < 0.01$; Fig. 2**D**). Moreover, MIA-CL2 but not MIA-CL1 offspring exhibited significantly decreased mean % PPI scores at adult age compared to adult CON-CL1 offspring (ANOVA: $F_{(2,33)} = 11.09$, $p < 0.001$; CON-CL1 versus MIA-CL2: $p < 0.001$; MIA-CL1 versus MIA-CL2: $p < 0.01$). Together, these findings show that the

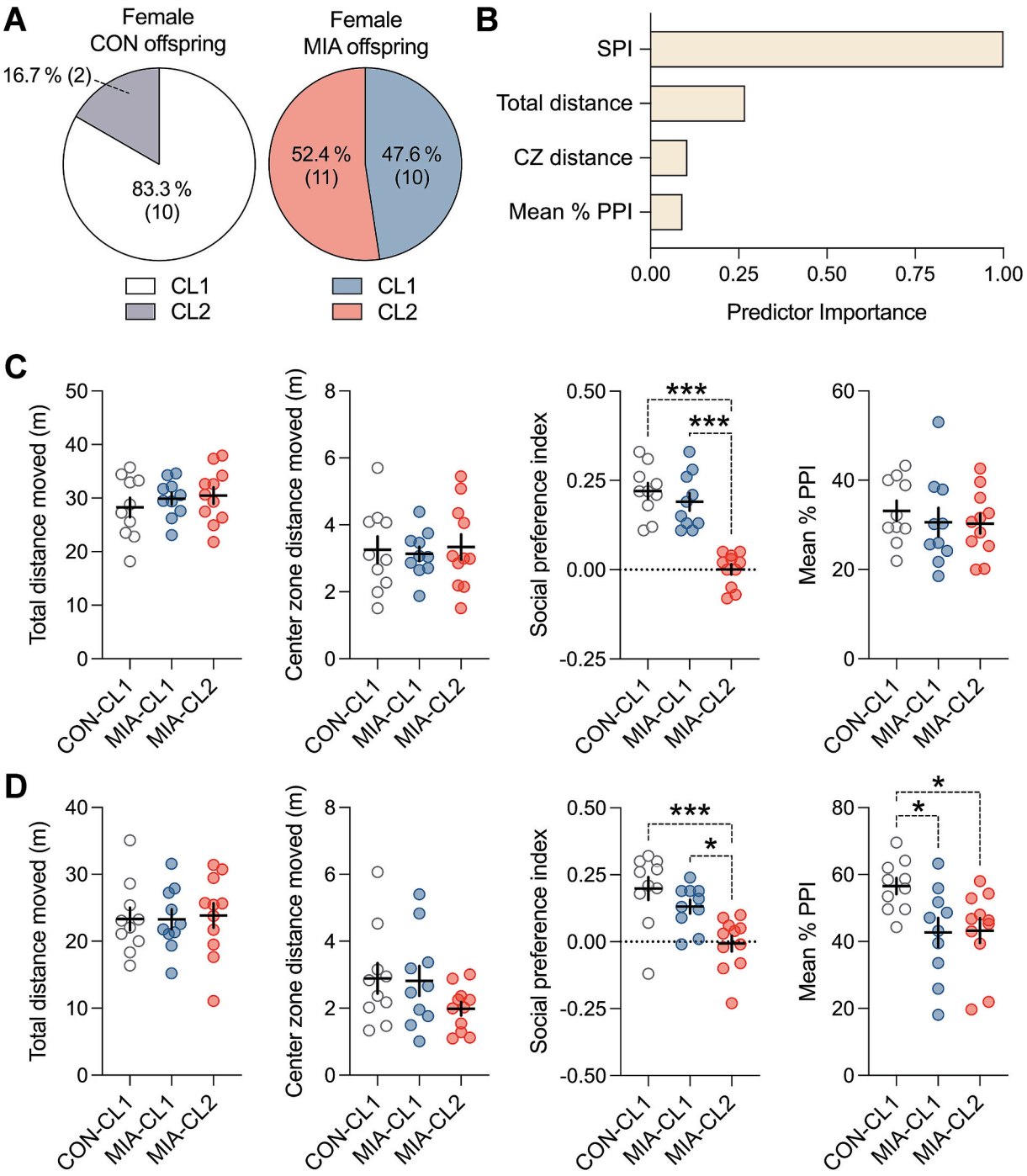

Fig. 3. Stratification of female offspring into subgroups with differing juvenile and adult behavioral profiles. A two-step cluster analysis incorporating juvenile behaviors (total distance and center zone [CZ] distance moved in the open field test; social preference index [SPI] in the social interaction test; mean percent prepulse inhibition [mean % PPI] in the PPI test) was performed to identify subgroups with differing behavioral profiles at juvenile age. The same subgroups were then retested in adulthood. **(A)** Distribution of juvenile female control (CON; $n = 12$) offspring and offspring exposed to maternal immune activation (MIA; $n = 21$) across the two clusters (CL1 and CL2) identified by two-step cluster analysis. The pie chart shows the cluster distribution (in percentages, %) for CON and MIA offspring, with numbers in brackets representing the number of female offspring in each cluster. **(B)** Relative predictor importance for cluster separation in juvenile female offspring. **(C)** Juvenile behaviors for female subgroups of CON and MIA offspring as identified by two-step cluster analysis. The plots show total distance moved (m), CZ distance moved (m), social preference index, and mean % PPI. **(D)** Adult behaviors for the same subgroups of offspring that were stratified based on juvenile behaviors. All scatter plots show individual mice with overlaid group means ± s.e.m; $^{*}p < 0.05$ and $^{***}p < 0.001$, based on Tukey's post-hoc test after ANOVA.

subgroup classification of male offspring at juvenile age predicts distinct behavioral outcomes in adulthood. However, the nature of the behavioral deficits in MIA subgroups varies between juvenile and adult stages, highlighting plasticity in subgroup-specific behavioral manifestations across adolescent maturation.

To further assess the predictive power of the juvenile subgroup classification in male offspring, we examined whether similar cluster memberships would emerge when conducting a cluster analysis of adult behavioral data. To this end, we incorporated the primary behavioral readouts from adult CON and MIA offspring into unsupervised two-step cluster analysis. Consistent with the juvenile data analysis, the adult cluster analysis revealed largely identical cluster ratios (**Suppl. Fig. S1A**), closely mirroring those observed in juvenile animals (Fig. 2**A**). In line with the juvenile cluster separation, adult MIA offspring in the MIA-CL2 subgroup exhibited a significant reduction in the social preference index and mean % PPI (**Suppl. Fig. S1C**). Notably, the majority of animals assigned to specific subgroups based on juvenile clustering maintained the same cluster membership when adult behavioral data were used for clustering (**Suppl. Fig. S1D**). In contrast to the juvenile clustering (Fig. 2**B**), however, the adult cluster separation was primarily predicted by the social preference index, followed by mean % PPI scores (**Suppl. Fig. S1B**).

3.2. Identification and follow-up of female subgroups with distinctive behavioral profiles at juvenile age

Similar to the males, unsupervised two-step cluster analysis in juvenile female offspring revealed two clusters (CL1 and CL2), with a silhouette coefficient above 0.55 indicating good cohesion and separation. Twenty females were assigned to CL1, while 13 fell into CL2. Among CON females, 83.3 % (10 of 12) were categorized as CL1 (Fig. 3**A**), compared to 47.6 % (10 of 21) of the female MIA offspring. The remaining 52.4 % (11 of 21) of juvenile female MIA mice were classified into CL2 (Fig. 3**A**). The distribution between CL1 and CL2 differed significantly between female CON and MIA groups ($\chi^2 = 4.08$, z $= 2.02$, $p < 0.05$).

Contrary to juvenile males (Fig. 2**A**), the social preference index in the social interaction test had the highest predictor importance for cluster separation in juvenile female offspring (Fig. 3**B**). The subsequent comparison of CON-CL1, MIA-CL1 and MIA-CL2 subgroups confirmed that juvenile MIA-CL2 females exhibited a significant decrease in the social preference index compared to the other subgroups (ANOVA: $F_{(2,28)} = 34.24$, $p < 0.001$; MIA-CL2 versus CON-CL1 or MIA-CL1: $p <$ 0.001; Fig. 3**C**). The subgroups did not differ in any other behavioral readouts measured at juvenile age (Fig. 3**C**).

After the stratification of juvenile females, the same subgroups were followed up into adulthood to assess the same behavioral parameters again. At adult age, female offspring in the MIA-CL2 subgroup continued to display a decrease in the social preference index compared to offspring in the CON-CL1 and MIA-CL1 subgroups (ANOVA: $F_{(2,28)} =$ 10.19, $p < 0.001$; MIA-CL2 versus CON-CL1: $p < 0.001$; MIA-CL2 versus MIA-CL1: $p < 0.05$; Fig. 3**D**). As adults, female MIA offspring from both subgroups exhibited a reduction in mean % PPI compared to adult female CON-CL1 offspring (ANOVA: $F_{(2,28)} = 4.70$, $p < 0.05$; CON-CL1 versus MIA-CL1 or MIA-CL2: $p < 0.05$; Fig. 3**D**). There were no differences between subgroups in terms of total or center zone distance moved in the open field (Fig. 3**D**). Thus, in agreement with the male data (Fig. 2), these findings demonstrate that the subgroup classification of female offspring at juvenile age predicts distinct behavioral outcomes in adulthood. Like in males (Fig. 2), the specificity of the behavioral deficits in the MIA subgroups varies between juvenile and adult stages, indicating that the subgroup-specific behavioral manifestations show a certain degree of plasticity across adolescent maturation.

To further assess the predictive power of the juvenile subgroup classification in female offspring, we investigated whether similar cluster memberships would emerge when conducting a cluster analysis of adult behavioral data. This cluster analysis revealed identical cluster ratios (**Suppl. Fig. S2A**), matching those observed when the cluster analysis was conducted with juvenile behavioral data (Fig. 3). Importantly, the majority of offspring assigned to specific subgroups based on juvenile clustering maintained the same cluster membership when adult behavioral data were used for clustering (**Suppl. Fig. S2D**). Consistent with the juvenile cluster separation, the social preference index had the highest predictor importance for the adult cluster separation, followed by mean % PPI scores (**Suppl. Fig. S2A**). Moreover, in line with the outcomes when cluster separation was performed using juvenile behavioral data (Fig. 3), adult females in the MIA-CL2 subgroup exhibited a significant reduction in the social preference index (**Suppl. Fig. S2C**). However, unlike the juvenile cluster separation, where both MIA subgroups showed reduced PPI as adults (Fig. 3**D**), only MIA-CL2 females showed a decrease in mean % PPI when clustering was conducted using adult behavioral data (**Suppl. Fig. S2C**).

3.3. Effects of repeated social interventions during adolescence on subgroup-specific behavioral trajectories

The longitudinal investigation of behavioral trajectories in MIA offspring from cohort 1 showed that deficits in juvenile sociability, either alone (female offspring; Fig. 3) or in conjunction with locomotor hyperactivity (male offspring; Fig. 2), preceded the emergence of subgroup-specific behavioral deficits in adulthood. Because social experience during peri-adolescent life is critical for establishing adult sociability and other behavioral and cognitive functions (Bicks et al., 2020; Leventhal and Morishita, 2024; Makinodan et al., 2012; Yamamuro et al., 2020), we investigated the effects of a repeated social intervention paradigm on subgroup-specific behavioral trajectories in male and female MIA offspring.

To this end, a second cohort of CON and MIA offspring was first subjected to a social interaction test on PND 21 to obtain juvenile sociability and locomotor activity scores. In males, this test revealed a subgroup of juvenile MIA offspring displaying a concomitant decrease and increase in sociability and locomotor activity, respectively (male MIA-CL2 subgroup; **Suppl. Fig. S3A**). In females, the juvenile social interaction test identified a subgroup of MIA offspring with low juvenile sociability in the absence of alterations in locomotor activity (female MIA-CL2 subgroup; **Suppl. Fig. S3A**). These male and female MIA subgroups were then subjected to repeated social interventions across adolescence, which involved weekly dyadic interactions between MIA-CL2 offspring and CON offspring with high juvenile sociability scores (**Suppl. Fig. S3**). The analysis of dyadic measures during each social intervention session revealed that nose-to-nose contacts and anogenital sniffing between MIA-CL2 offspring and social partners increased and decreased, respectively, over successive sessions in both males (**Suppl. Fig. S4A**) and females (**Suppl. Fig. S4B**). As a negative control intervention, stratified CON or MIA offspring were subjected to a sham procedure for the same duration without a social partner (Fig. 1).

In male offspring, the social intervention did not improve the adult deficits in sociability and temporal order memory. Hence, compared to adult male CON-CL1 and MIA-CL1 offspring, which received sham control interventions only, adult male MIA-CL2 offspring displayed a significant reduction in the social preference index (ANOVA: $F_{(3,32)} =$ 9.61, $p < 0.001$; CON-CL1 or MIA-CL1 versus MIA-CL2 with or without social stimulation: $p < 0.01$) and temporal order memory index (ANOVA: $F_{(3,32)} = 5.73$, $p < 0.01$; CON-CL1 or MIA-CL1 versus MIA-CL2 with or without social stimulation: $p < 0.05$) regardless of whether they were subjected to social interventions during adolescence or not (Fig. 4**A**). However, social stimulation during adolescence prevented the subsequent emergence of adult PPI deficits in male MIA-CL2 offspring. As shown in Fig. 4**A**, only MIA-CL2 offspring that did not receive the social intervention displayed a significant reduction in mean % PPI (ANOVA: $F_{(3,32)} = 5.35$, $p < 0.01$; MIA-CL2 without social stimulation versus all other subgroups: $p < 0.05$). In contrast, MIA-CL2 offspring that

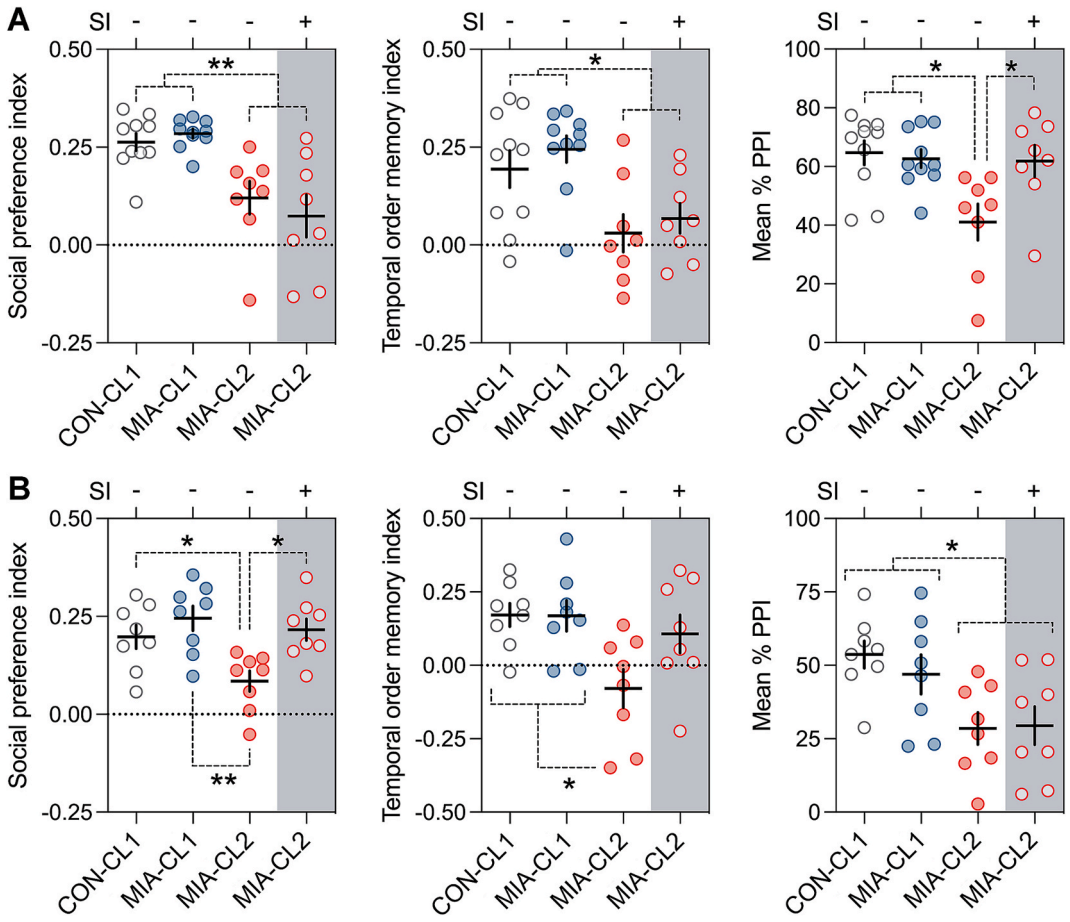

Fig. 4. Effects of repeated social intervention during adolescence on subgroup-specific behavioral and cognitive functions in adulthood. Offspring exposed to maternal immune activation (MIA) were stratified into subgroups (MIA-CL1 and MIA-CL2) based on juvenile sociability and locomotor activity scores (see *Suppl. Fig. S3*). A subset of MIA-CL2 offspring underwent repeated social intervention (SI +) during adolescence and were compared to MIA-CL2 offspring that did not receive social intervention (SI −). CON-CL1 and MIA-CL1 offspring that did not receive social intervention (SI −) served as negative control groups. **(A)** Behavioral and cognitive outcomes in male offspring. Scatter plots show individual mice with overlaid group means ± s.e.m. for the social preference index in the social interaction test (left), temporal order memory index in the temporal order memory test (middle), and mean % PPI in the sensorimotor gating test (right). *$p < 0.05$ and **$p < 0.01$, based on Tukey's post hoc test following ANOVA. **(B)** Behavioral and cognitive outcomes in female offspring. Scatter plots show individual mice with overlaid group means ± s.e.m. for the social preference index in the social interaction test (left), temporal order memory index in the temporal order memory test (middle), and mean % PPI in the sensorimotor gating test (right). *$p < 0.05$ and **$p < 0.01$, based on Tukey's post hoc test following ANOVA.

received the intervention displayed PPI levels comparable to those of CON-CL1 or MIA-CL1 offspring (Fig. 4**A**).

Contrary to males, the social intervention prevented the adult emergence of deficits in sociability and temporal order memory in female MIA-CL2 offspring. As shown in Fig. 4**B, a** significant reduction in the social preference index (ANOVA: $F_{(3,28)} = 5.90$, $p < 0.01$; MIA-CL2 without social stimulation versus all other subgroups: $p < 0.05$) and the temporal order memory index (ANOVA: $F_{(3,28)} = 4.35$, $p < 0.05$; MIA-CL2 without social stimulation versus CON-CL1 or MIA-CL1 offspring: $p < 0.05$) was observed only in adult female MIA-CL2 offspring that did not receive social stimulation, whereas those that underwent social intervention sessions during adolescence did not exhibit these deficits. However, the social intervention in MIA-CL2 females failed to prevent the deficit in PPI at adult age (Fig. 4**B**). Indeed, adult female MIA-CL2 offspring displayed a significant reduction in mean % PPI regardless of whether they were subjected to social interventions during adolescence or not (ANOVA: $F_{(3,28)} = 4.59$, $p < 0.01$; CON-CL1 or MIA-CL1 versus MIA-CL2 with or without social stimulation: $p < 0.05$; Fig. 4**B**).

3.4. Effects of repeated social interventions during adolescence on subgroup-specific changes in oxytocinergic and dopaminergic markers

Given that repeated social stimulation during adolescence resulted in subgroup- and sex-specific effects on adult behavioral and cognitive functions in MIA offspring (Fig. 4), we next examined whether these effects were associated with changes in the expression of oxytocinergic and dopaminergic markers in cortical and subcortical regions. To this end, we collected brain tissue from the behaviorally tested cohort of animals (cohort 2; Fig. 4) and quantified OXT-positive cells in the PVN, as well as OXTR and TH immunoreactivity in various cortical and subcortical brain areas.

In male offspring, there were no differences between the different subgroups of CON and MIA offspring in terms of the density and intensity of immunoreactive OXT cells in the PVN (Fig. 5**A,B**). The density of OXT-immunoreactive PVN cells did also not differ between the female subgroups of CON and MIA offspring (Fig. 5**C**). However, MIA-CL2 females receiving no social stimulation during adolescence displayed a significant reduction in the intensity of OXT immunoreactivity compared to CON-CL1 and MIA-CL2 females receiving social stimulation (ANOVA: $F_{(3,28)} = 4.29$, $p < 0.05$; MIA-CL2 without social stimulation versus CON-CL1 or MIA-CL2 with social stimulation: $p < 0.05$; Fig. 5**C**). These findings show that social stimulation during adolescence prevents

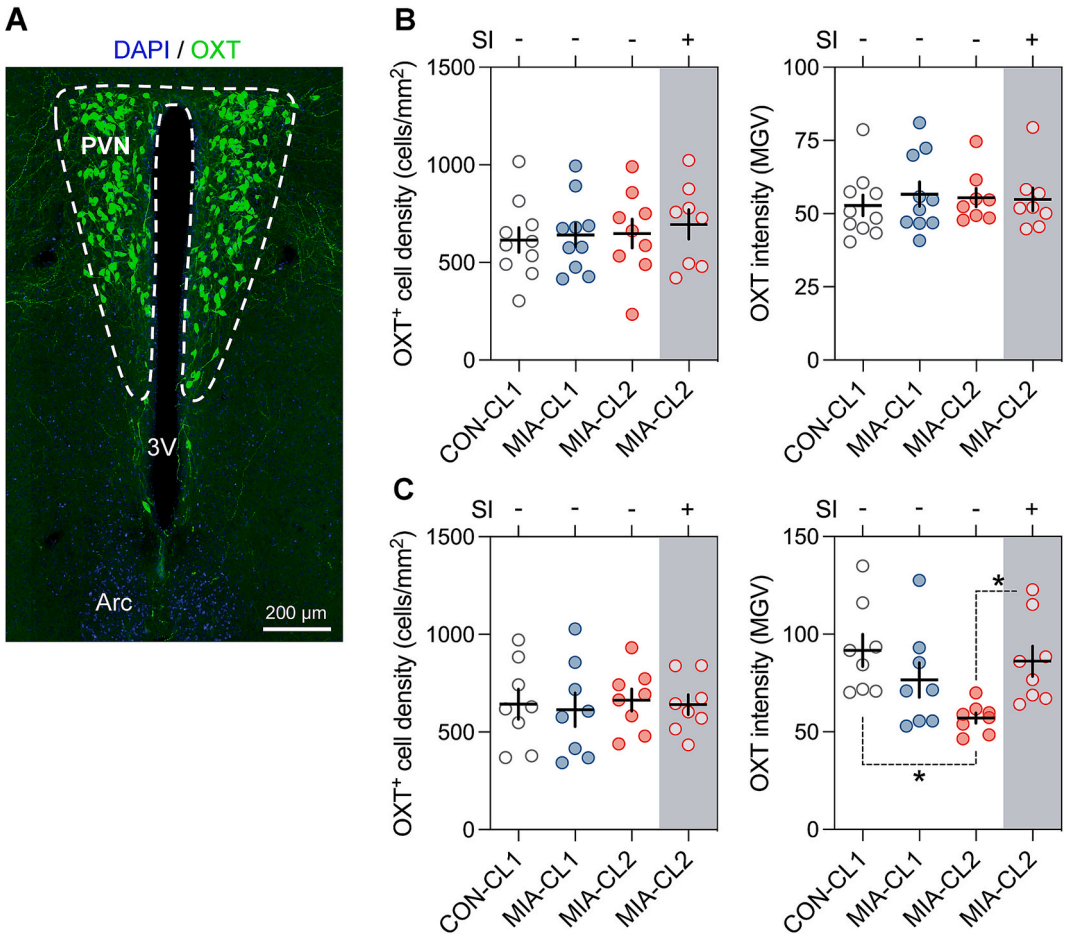

Fig. 5. Effects of repeated social intervention during adolescence on subgroup-specific alterations in the density and intensity of oxytocin (OXT)-immunoreactive cells in the paraventricular nucleus (PVN) of the hypothalamus. Offspring exposed to maternal immune activation (MIA) were stratified into subgroups (MIA-CL1 and MIA-CL2) based on juvenile sociability and locomotor activity scores (see *Suppl. Fig. S3*). A subset of MIA-CL2 offspring underwent repeated social intervention (SI +) during adolescence and were compared to MIA-CL2 offspring that did not receive social intervention (SI −). CON-CL1 and MIA-CL1 offspring that did not receive social intervention (SI −) served as negative control groups. **(A)** The photomicrograph shows a representative immunofluorescence stain using anti-OXT antibody, taken at the level of the PVN. 4′,6-diamidino-2-phenylindole (DAPI; blue) was used as a counterstain to visualize cell nuclei. 3V, third ventricle; Arc, arcuate nucleus. **(B)** OXT^+ cell density (cells/mm^2) and OXT intensity (mean gray value, MGV) in male offspring. **(C)** OXT^+ cell density (cells/mm^2) and OXT intensity (MGV) in female offspring. All scatter plots show individual mice with overlaid group means ± s.e.m.; *$p < 0.05$, based on Tukey's post hoc test following ANOVA.

the adult emergence of reduced OXT immunoreactivity in the PVN of MIA-CL2 females.

In line with these findings, there were also sex- and subgroup-specific effects on OXTR immunoreactivity. In males, OXTR immunoreactivity did not differ between subgroups in any of the brain regions examined (Fig. 6**A,B**; **Suppl. Fig. S5A**). There were also no differences between the different subgroups of female CON and MIA offspring in terms of OXTR immunoreactivity in hippocampal subregions (**Suppl. Fig. S5B**). In female MIA-CL2 offspring receiving no social stimulation during adolescence (Fig. 6**C**), however, OXTR immunoreactivity was significantly reduced in the mPFC (ANOVA: $F_{(3,28)} = 3.41$, $p < 0.05$; MIA-CL2 without social stimulation versus CON-CL1: $p < 0.05$), BLA (ANOVA: $F_{(3,28)} = 3.98$, $p < 0.05$; MIA-CL2 without social stimulation versus CON-CL1: $p < 0.05$), and CeA (ANOVA: $F_{(3,28)} = 3.22$, $p < 0.05$; MIA-CL2 without social stimulation versus CON-CL1: $p < 0.05$). These reductions were not present in MIA-CL2 offspring subjected to the social intervention (Fig. 6**C**), indicating that social stimulation during adolescence attenuated the emergence of OXTR deficits in female MIA-CL2 offspring.

In addition to the observed alterations in oxytocinergic markers, there were also sex- and subgroup-specific effects on TH immunoreactivity, with male offspring being more affected than females (Fig. 7). Specifically, MIA-CL2 males exhibited significantly reduced TH immunoreactivity in the mPFC (ANOVA: $F_{(3,32)} = 5.75$, $p < 0.01$; MIA-CL2 with or without social stimulation versus CON-CL1 or MIA-CL1: $p < 0.05$) and NAc (ANOVA: $F_{(3,32)} = 4.88$, $p < 0.01$; MIA-CL2 with or without social stimulation versus CON-CL1 or MIA-CL1: $p < 0.05$) regardless of whether they were subjected to the social intervention or not (Fig. 7**A,B**). By contrast, MIA-CL2 males not receiving social stimulation during adolescence exhibited significantly increased TH immunoreactivity in the CPu, an effect that was normalized by the social intervention (ANOVA: $F_{(3,32)} = 4.55$, $p < 0.01$; MIA-CL2 without social stimulation versus all other subgroups: $p < 0.05$; Fig. 7**B**). In females, TH immunoreactivity did not differ between subgroups in any of the brain regions examined (Fig. 7**C**).

4. Discussion

Using a mouse model of poly(I:C)-induced MIA, our study demonstrates that MIA offspring can be stratified into subgroups exhibiting distinct behavioral profiles as early as the juvenile stage. This early behavioral divergence was found to be sex-dependent. While female MIA offspring could be stratified based on the presence of absence of deficits in juvenile sociability, the strongest predictor of subgroup separation in juvenile males was the presence or absence of increased locomotor and exploratory activity, either in isolation or co-occurring with juvenile social impairments. These findings extend previous studies

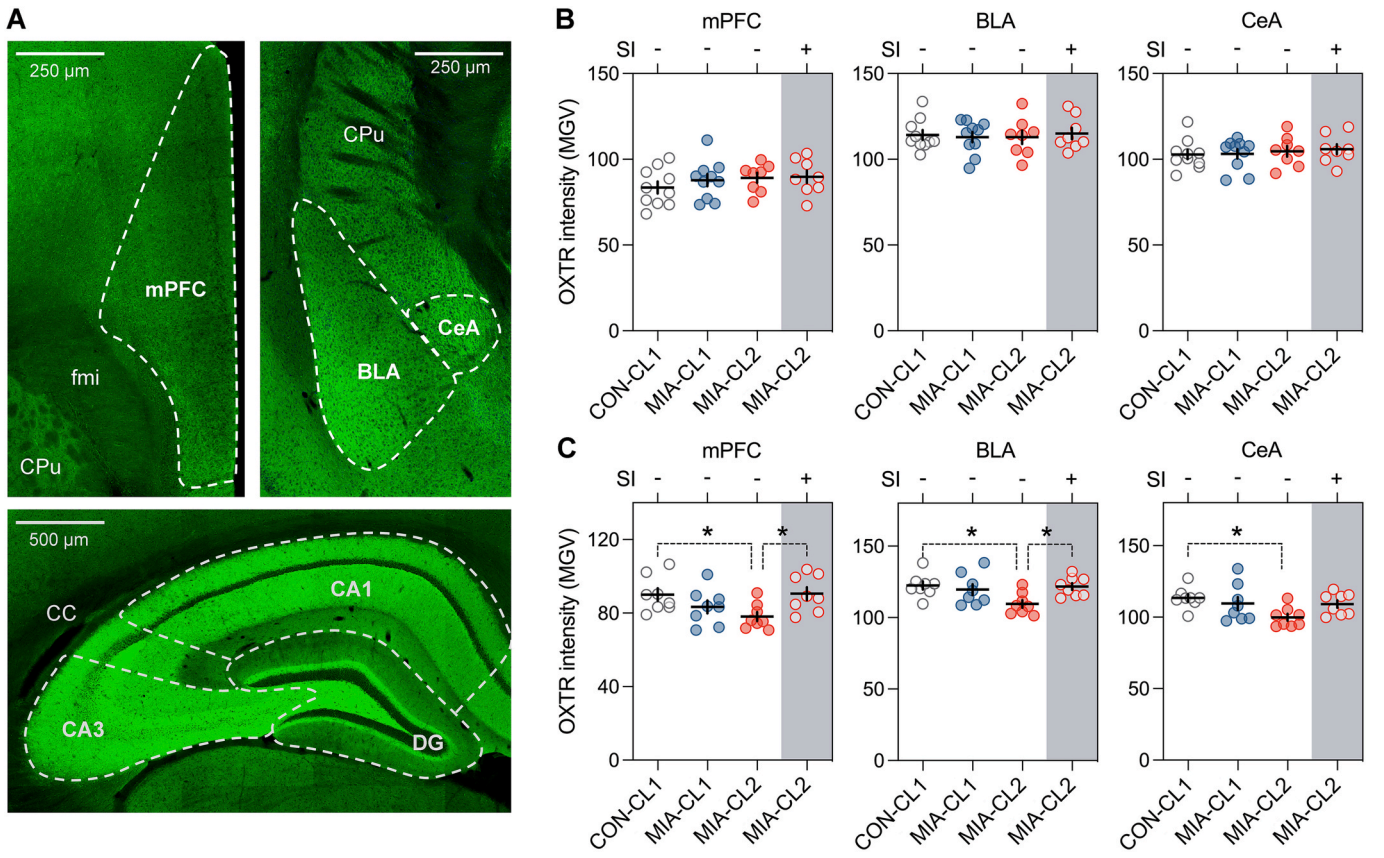

Fig. 6. Effects of repeated social intervention during adolescence on subgroup-specific alterations in the intensity of oxytocin receptor (OXTR) immunoreactivity in cortical and subcortical brain areas of interest. Offspring exposed to maternal immune activation (MIA) were stratified into subgroups (MIA-CL1 and MIA-CL2) based on juvenile sociability and locomotor activity scores (see *Suppl. Fig. S3*). A subset of MIA-CL2 offspring underwent repeated social intervention (SI +) during adolescence and were compared to MIA-CL2 offspring that did not receive social intervention (SI −). CON-CL1 and MIA-CL1 offspring that did not receive social intervention (SI −) served as negative control groups. **(A)** The photomicrographs show representative immunofluorescence stains using anti-OXTR antibody, taken at the level of the regions of interest (as indicted by the dashed lines). OXTR immunoreactivity was analyzed in the medial prefrontal cortex (mPFC), basolateral amygdala (BLA), and central amygdala (CeA), as well as in the CA1 and CA3 subfields and dentate gyrus (DG) region of the hippocampus. CC, corpus callosum; CPu, caudate putamen; fmi, forceps minor of the corpus callosum. **(B)** OXTR intensity (mean gray value, MGV) in the mPFC, BLA, and CeA of male offspring. **(C)** OXTR intensity (MGV) in the mPFC, BLA, and CeA of female offspring. All scatter plots show individual mice with overlaid group means ± s.e.m.; $*p < 0.05$, based on Tukey's post hoc test following ANOVA. All data for CA1, CA3, and DG are shown in *Suppl. Fig. S5*.

showing that MIA can lead to variable behavioral and cognitive outcomes at adult age (Herrero et al., 2023; Lorusso et al., 2022; Mueller et al., 2021; Schaer et al., 2024). Importantly, our data offer a novel perspective on the early manifestation of MIA-induced variability, suggesting that susceptible and resilient phenotypes can be identified prior to adulthood.

We found consistent subgroup-specific effects of MIA in both cohort 1 and cohort 2, despite differences in the timing of behavioral assessments. Specifically, cohort 1 was tested starting at PND 28, whereas cohort 2 was first tested at PND 21. Importantly, similar subgroups emerged in both cohorts based on overlapping behavioral measures, namely the social preference index and locomotor activity scores. Thus, comparable behavioral profiles were detectable across cohorts, even though the timing of testing relative to weaning differed. While we cannot entirely rule out the possibility that the results might have differed if testing had been conducted later in adolescence, we consider it unlikely that such differences would stem from enduring effects of maternal care. Rather, any age-related variation in subgroup stratification would more likely reflect the ongoing maturation of the brain during this developmental window.

This interpretation is further supported by our longitudinal data from cohort 1. Although MIA offspring could be stratified into distinct subgroups based on behavioral profiles at the juvenile stage, these profiles changed over the course of adolescent maturation, resulting in adult behavioral patterns that differed from those seen in juvenile life. This was particularly evident in the CL2 subgroup of MIA males, which showed increased locomotor activity as juveniles but no longer displayed this behavior in adulthood. Instead, adult males in this subgroup showed reduced PPI concomitant with social deficits. In female MIA offspring, the juvenile social deficits remained present into adulthood within the same subgroup, but these animals also began to show reduced PPI as adults. Thus, in both sexes, adult CL2 MIA offspring exhibited impairments in sociability and PPI, a finding that aligns with previous studies indicating that susceptible male and female MIA offspring develop comparable deficits in these domains when reaching adulthood (Herrero et al., 2023; Mueller et al., 2021; Schaer et al., 2024). Importantly, our longitudinal study design allowed us to reveal that, despite similar behavioral outcomes in adulthood, the developmental trajectories leading to these adult phenotypes were sex-dependent.

Of note, even though the behavioral profiles defining juvenile and adult MIA subgroups were not identical, our findings strongly support the accuracy of predicting adult subgroup membership based on juvenile clustering. To evaluate the predictive strength of the juvenile classifications, we conducted an additional cluster analysis using only the adult behavioral data. This analysis revealed cluster ratios that were largely identical to those identified in the juvenile stage and showed a high degree of consistency in individual cluster membership across development. These results indicate that juvenile subgroup

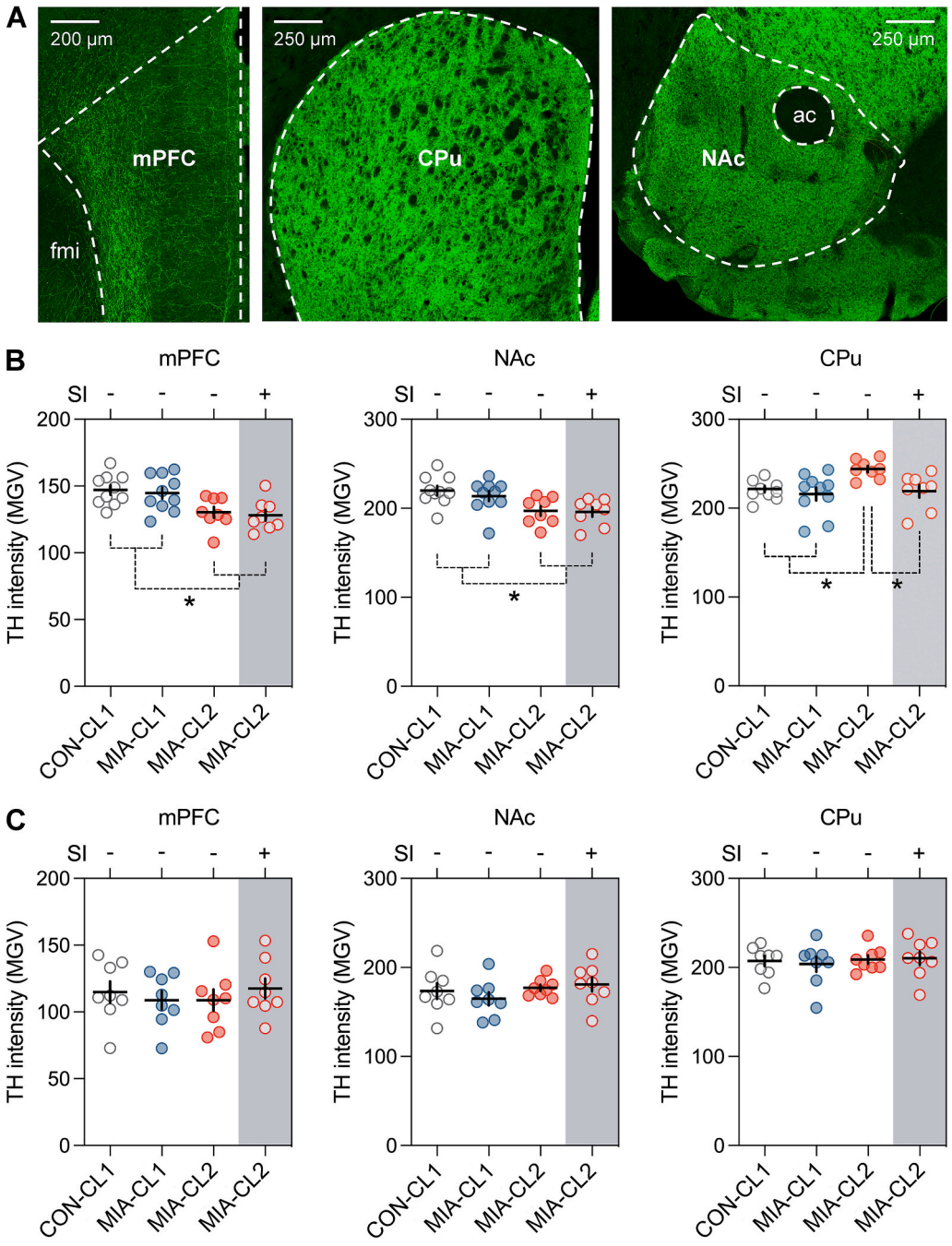

Fig. 7. Effects of repeated social intervention during adolescence on subgroup-specific alterations in the intensity of tyrosine hydroxylase (TH) immunoreactivity in cortical and subcortical brain areas of interest. Offspring exposed to maternal immune activation (MIA) were stratified into subgroups (MIA-CL1 and MIA-CL2) based on juvenile sociability and locomotor activity scores (see *Suppl. Fig. S3*). A subset of MIA-CL2 offspring underwent repeated social intervention (SI +) during adolescence and were compared to MIA-CL2 offspring that did not receive social intervention (SI −). CON-CL1 and MIA-CL1 offspring that did not receive social intervention (SI −) served as negative control groups. **(A)** The photomicrographs show representative immunofluorescence stains using anti-TH antibody, taken at the level of the regions of interest (as indicted by the dashed lines). TH immunoreactivity was analyzed in the medial prefrontal cortex (mPFC), caudate putamen (CPu), and nucleus accumbens (NAc). ac, anterior commissure, fmi, forceps minor of the corpus callosum. **(B)** TH intensity (mean gray value, MGV) in the mPFC, NAc, and CPu of male offspring. **(C)** TH intensity (MGV) in the mPFC, NAc, and CPu of female offspring. All scatter plots show individual mice with overlaid group means ± s.e.m.; $*p < 0.05$, based on Tukey's post hoc test following ANOVA.

classifications reliably predict the emergence and/or persistence of specific behavioral deficits into adulthood. Importantly, while some behavioral features shifted over time, the overall distinction between MIA subgroups remained largely stable. Thus, our study suggests that juvenile behavioral data provide a reliable and informative basis for anticipating the extent to which MIA offspring can be stratified into resilient and susceptible subgroups in adult life.

Based on these findings, we were also interested in examining whether the subgroup-specific behavioral trajectories could be modified by an early behavioral intervention implemented during juvenile to early adult life. Our longitudinal cohort of offspring suggested that deficits in juvenile sociability, either alone (in female offspring) or in conjunction with locomotor hyperactivity (in male offspring), preceded the emergence of subgroup-specific behavioral deficits in adulthood.

Therefore, we investigated the effects of a repeated social intervention paradigm on these trajectories in male and female MIA offspring. We found that the social intervention had highly sex-specific effects on adult behavioral and cognitive functions. In male MIA offspring displaying juvenile deficits in sociability and hyperactivity, the intervention did not improve adult deficits in sociability or temporal order memory, but it did prevent the adult emergence of PPI impairments. By contrast, in female MIA offspring with juvenile social deficits, the social intervention improved adult deficits in sociability and temporal order memory, while it failed to normalize PPI impairments. Together, these data identify a window of plasticity during which targeted interventions can modulate abnormal maturational trajectories, ultimately mitigating the long-term effects of MIA. This suggests that early interventions may offer promising avenues for mitigating the developmental consequences of MIA, potentially providing therapeutic strategies to reduce the severity of associated behavioral and cognitive impairments across the lifespan. However, these findings also underscore the complex nature of intervention outcomes and highlight the need to develop tailored approaches that specifically account for the sex differences among MIA-exposed offspring.

The distinct behavioral profiles observed between male and female juvenile animals, as well as the sex-specific parameters driving cluster formation, may reflect underlying neurodevelopmental processes that diverge by sex. It is well established that male and female brains undergo differential trajectories of maturation, influenced by both hormonal and genetic factors (McCarthy and Wright, 2017). For instance, sex hormones such as testosterone and estradiol play critical roles in shaping neural circuits involved in social behavior, affect regulation, and sensorimotor processing during sensitive developmental windows (McCarthy and Wright, 2017; Schulz and Sisk, 2016). One key region implicated in sex-specific effects is the PFC, which undergoes prolonged maturation into adolescence and early adulthood, with notable differences in timing and structure between sexes (Chini and Hanganu-Opatz, 2021; Gogtay et al., 2004; Kaczkurkin et al., 2019). The PFC plays a central role in top-down regulation of motor output, impulse control, and social behavior, and its development is closely linked to changes in locomotor activity and exploratory behavior (Chambers et al., 2003; Kolb et al., 2012; Schalbetter et al., 2022). Therefore, MIA-induced delays or differences in PFC maturation (Crum et al., 2017; Vernon et al., 2015; Vlasova et al., 2021) could contribute to sex- and subgroup-specific locomotor profiles identified in our study. Taken together, the sex-specific behavioral clusters may, at least in part, reflect differential maturation of frontocortical circuits involved in locomotor activity, sensorimotor processing, and social behaviors.

Consistent with this notion, the social intervention produced sex-specific changes in oxytocinergic and dopaminergic markers across cortical and subcortical brain regions. In male MIA offspring, the CL1 and CL2 subgroups did not differ with regards to the oxytocinergic markers, and these markers remained unchanged following the social intervention. However, male MIA CL2 offspring exhibited increased TH immunoreactivity in the CPu, which was normalized by the intervention. In contrast, reduced TH immunoreactivity in the mPFC and NAc observed in male MIA CL2 offspring was not rescued by the social intervention. Unlike males, the female CL1 and CL2 MIA subgroups did not differ in TH immunoreactivity but did show significant differences in oxytocinergic markers. More specifically, female MIA CL2 offspring displayed a reduction in the intensity of OXT-expressing cells in the PVN and in OXTR immunoreactivity in the mPFC, BLA, and CeA, all of which were normalized by the social intervention. Taken together, our findings suggest that the differential effects of the social intervention in male and female MIA offspring result from the interplay of several factors. Specifically, our data highlight that the neurobiological substrates underlying MIA-induced deficits vary not only by sex but also by the presence or absence of overt behavioral abnormalities during juvenile life, underscoring the importance of tailoring early interventions to the sex-specific needs and vulnerabilities of populations at risk for (MIA-induced) neurodevelopmental disorders.

In a broader context, our findings are consistent with and corroborate the hypothesis that appropriate social functioning throughout infancy and adolescence plays a critical role in shaping behavioral and cognitive outcomes later in life. For instance, social deprivation during the juvenile period (PND 21–35), but not during early adulthood (PND > 60), has been shown to induce long-term cognitive impairments and white matter abnormalities in mice (Makinodan et al., 2012). Furthermore, social interaction during the juvenile stage is essential for the proper maturation of prefrontal interneurons and for the development of adult cognitive functions mediated by the PFC in mice (Bicks et al., 2020). Similarly, the maturation of prefrontal-thalamic circuits in mice depends on juvenile social experience, and its disruption results in persistent brain and behavioral abnormalities in adulthood (Yamamuro et al., 2020). As in rodents, social abilities in humans begin to develop during infancy but continue to mature throughout childhood and adolescence (Blakemore, 2012). This gradual development mirrors the prolonged maturation of the so-called “social brain”, a network of interconnected cortical and subcortical areas responsible for social behavior and cognition (Blakemore, 2012). During this period, children with low levels of social engagement are at increased risk of developing mental health issues in later life (Andrews et al., 2021). Conversely, consistent social engagement and prosocial behavior from middle childhood through late adolescence are associated with a reduced risk of mental health problems, including externalizing symptoms in boys and internalizing symptoms in girls during early adulthood (Flynn et al., 2015). Collectively, these findings highlight the pivotal role of juvenile social experience in sculpting brain circuits to meet the evolving cognitive and emotional demands associated with the transition from adolescence to adulthood. As a result, the absence of adequate social input during this critical developmental window may lead to enduring behavioral and cognitive deficits implicated in psychiatric disorders (Larsen and Luna, 2018). Our findings align with these observations, revealing that low juvenile sociability in susceptible MIA offspring is associated with the emergence of multiple behavioral and cognitive impairments in adulthood, effects that can be partially ameliorated by repeated social interventions during the periadolescent period.

Our data also indicate that male offspring are more affected than female offspring in terms of how MIA influences the central dopaminergic systems. Consistent with our findings, a previous study using the poly(I:C)-induced MIA model in mice found that male, but not female, MIA offspring displayed increased TH immunoreactivity in the CPu at adult age (Meyer et al., 2008a). Likewise, it was previously shown that poly(I:C)-induced MIA in rats affects the electrophysiological properties of midbrain dopamine neurons in male, but not female, offspring (De Felice et al., 2019; Lecca et al., 2019; Santoni et al., 2022). More recently, it was demonstrated that catechol-O-methyltransferase (*Comt*) mRNA levels were increased in the substantia nigra of male, but not female, rat offspring exposed to poly(I:C)-induced MIA (Debs et al., 2024). On the other hand, our data suggest that female offspring are more affected than males in terms of MIA-induced changes in the central OXT system. Interestingly, in line with our data, Zhao and colleagues found a concomitant reduction in hippocampal OXTR expression and sociability in female, but not male, mice prenatally exposed to poly(I:C), both of which were normalized by environmental enrichment (Zhao et al., 2021b). The same study further showed that while male MIA offspring exhibited a similar deficit in social interaction, this deficit was not accompanied by reduced, but rather increased, hippocampal OXTR levels. Hence, the study by Zhao and colleagues highlighted opposite effects of MIA on OXTR expression in male and female mice, despite both sexes exhibiting a similar deficit in sociability (Zhao et al., 2021b). These findings are consistent with our data presented here, indicating that similar behavioral and cognitive deficits in male and female MIA offspring are likely mediated through partially different mechanisms. On speculative grounds, alterations in dopaminergic functions may more readily contribute to the deficits in male MIA offspring, whereas

changes in the central OXT system may be pathologically more relevant in female MIA offspring.

We recognize several limitations in our study. First, while the longitudinal design in cohort 1 allowed us to approximate the behavioral trajectories of resilient and susceptible MIA offspring, the absence of more frequent monitoring or a broader behavioral repertoire may have missed important transient phases of behavioral development. However, it is worth noting that more frequent longitudinal testing or the use of a more comprehensive behavioral battery might impose additional stress on the animals (Võikar et al., 2004), potentially confounding the outcomes in adulthood. Second, although the social intervention paradigm showed promising results in modulating the behavioral trajectories of susceptible MIA offspring, the exact mechanisms underlying these effects remain unknown and require further investigation. Specifically, while we explored initial candidates in the oxytocinergic and dopaminergic systems, our study was not designed to examine how the social intervention paradigm might influence the maturation of these systems in detail. Given that the social intervention paradigm likely affects a broad range of hormones, neurochemistry, and emotional states, future work focusing on more specific interventions could help elucidate the mechanisms underlying the plasticity of these behavioral trajectories over the course of postnatal maturation. Third, we did not assess the stage of the estrous cycle in our study. While hormonal fluctuations across the cycle can impact certain behaviors, previous studies suggest that various behavioral profiles, including those assessed by the open field, social interaction, and PPI tests, remain relatively consistent throughout the estrous cycle in female C57BL/6 mice (Meziane et al., 2007; Plappert et al., 2005; Zhao et al., 2021a). These findings suggest that the estrous cycle may not be a major contributor to behavioral variability in female C57BL/6J mice. However, we acknowledge that future studies would benefit from monitoring estrous cycle stages to better delineate the role of hormonal fluctuations in sex-specific behavioral responses, especially in the context of neurodevelopmental perturbations such as MIA.

Despite these limitations, we conclude that offspring born to MIA-exposed mothers can be stratified into distinct behavioral subgroups as early as the juvenile stage, with subsequent risk and resilience trajectories being sex-dependent. In male offspring, abnormally high locomotor activity at the juvenile stage precedes the adult emergence of deficits in sociability and sensorimotor gating. Conversely, in female offspring, low sociability observed during the juvenile period persists into adulthood. Additionally, our study supports the hypothesis that social engagement during periadolescent maturation exerts sex-dependent protective effects against the later emergence of behavioral and cognitive impairments in susceptible MIA offspring.

CRediT authorship contribution statement

Ron Schaer: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Nicole Wenger:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Felisa Herrero:** Writing – review & editing, Investigation, Formal analysis. **Tina Notter:** Writing – review & editing, Supervision, Methodology, Funding acquisition. **Urs Meyer:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2025.06.040.

Data availability

Data will be made available on request.

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