Exercise Reverses Behavioral and Synaptic Abnormalities after Maternal Inflammation

Highlights

- MIA causes behavioral and synaptic abnormalities in the offspring
- Microglia-dependent synaptic engulfment is impaired by MIA
- Voluntary running in adulthood ameliorates MIA-induced abnormalities
- Voluntary running stimulates microglia-mediated engulfment of surplus synapses

In Brief

Andoh et al. find that maternal immune activation (MIA) causes autism spectrum disorder (ASD)-like behaviors and synaptic surplus in the offspring in mice. Voluntary running normalizes synaptic density and ameliorates abnormal behaviors even after the onset of ASD-like behaviors, probably by boosting synaptic engulfment by microglia.

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Exercise Reverses Behavioral and Synaptic Abnormalities after Maternal Inflammation

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SUMMARY

Abnormal behaviors in individuals with neurodevelopmental disorders are generally believed to be irreversible. Here, we show that voluntary wheel running ameliorates the abnormalities in sociability, repetitiveness, and anxiety observed in a mouse model of a neurodevelopmental disorder induced by maternal immune activation (MIA). Exercise activates a portion of dentate granule cells, normalizing the density of hippocampal CA3 synapses, which is excessive in the MIA-affected offspring. The synaptic surplus in the MIA offspring is induced by deficits in synapse engulfment by microglia, which is normalized by exercise through microglial activation. Finally, chemogenetically induced activation of granule cells promotes the engulfment of CA3 synapses. Thus, our study proposes a role of voluntary exercise in the modulation of behavioral and synaptic abnormalities in neurodevelopmental disorders.

INTRODUCTION

Physical exercise has attracted attention as a potential therapeutic strategy for abnormal behaviors in neurodevelopmental disorders such as autism spectrum disorders (ASDs) (Pan, 2010; Anderson-Hanley et al., 2011), but the cellular and molecular mechanisms for how exercise ameliorates the symptoms remain unrevealed. Previous studies reported that exercise may help improve cognitive performance in humans and rodents (Aberg et al., 2009; Gomes da Silva et al., 2012), probably by enhancing the production of neurotrophic factors and neurogenesis (de Almeida et al., 2013; Pereira et al., 2007). However, the question of whether exercise modifies synaptic connections, which are fundamental structures for brain function, remains unanswered.

The frequency of neurodevelopmental disorders is positively correlated with maternal immune activation (MIA), which is induced by viral infection during pregnancy (Knuesel et al., 2014), and several studies have reported that deficits in synaptic function and structure underlie the pathogenesis of neurodevelopmental disorders (Koyama and Ikegaya, 2015). Specifically, the disruption of synapse excitatory versus inhibitory (E/I) balance (Rubenstein and Merzenich, 2003; Yizhar et al., 2011; Tyzio et al., 2014) and the increased spine or synapse density (Tang et al., 2014; Jawaid et al., 2018; Andoh et al., 2016) are shared pathological features between ASD patients and ASD animal models. These findings motivated us to examine whether voluntary exercise in adulthood reverses the behavioral abnormalities and affects synaptic properties in mouse offspring prenatally subjected to MIA (MIA offspring).

To test this idea, we focused on the hippocampus, because physical exercise has been suggested to activate neurons in the hippocampal dentate gyrus (Clark et al., 2011) and sociability, impairment of which is a major symptom of ASD, is partly mediated by the hippocampus (Hitti and Siegelbaum, 2014; Okuyama et al., 2016).

In the present study, we examined the role of microglia, the resident immune cells in the brain, in synaptic deficits in MIA offspring. Microglia continuously survey the brain environment, monitoring and modulating synaptic structure and function; microglia prune synapses in the lateral geniculate nucleus (LGN) and hippocampal CA1 (Schafer et al., 2012; Paolicelli et al., 2011) during development. We examined whether MIA induces synaptic deficits by affecting synaptic pruning by microglia, because MIA has been suggested to affect microglial properties such as number, morphology, and cytokine expression (Zhu et al., 2014; Hui et al., 2018; Mattei et al., 2017), possibly leading to abnormalities in microglial functions, including their phagocytic capacities (Giovanoli et al., 2013; Fernández de Cossio et al., 2017).

RESULTS

Exercise in Adulthood Ameliorates MIA-Associated Behaviors

We used offspring from pregnant mice intraperitoneally injected with the synthetic double-stranded RNA polyinosinic:polycytidylic acid (poly[I:C]), on embryonic days 12.5 and 17.5 to model MIA induced by viral infection during pregnancy (Malkova et al., 2012; Navaux et al., 2013). Consistent with previous reports, we found that MIA offspring at postnatal day 60 exhibited deficits...
in social interaction using the three-chamber assay (Figure 1A)
and repetitive behaviors by analyzing self-grooming time (Figure 1B). Because MIA is likely associated with anxiety in offspring (Ulmer-Yaniv et al., 2018), we also performed the novelty-suppressed feeding test and found that the MIA offspring displayed enhanced anxiety (Figure 1C). Next, we examined whether the MIA-associated behaviors could be reversed by adult voluntary exercise. For this purpose, we placed a running wheel in the cage of MIA offspring from P30 to P60. After a month of voluntary running exercise, all observed MIA-associated behaviors were ameliorated (Figures 1A–1C). We confirmed that neither MIA treatment nor exercise affected basal motility (total distance: control, 3,621.29 ± 143.69 cm; MIA, 3,472.24 ± 115.58 cm; MIA + exercise, 3,225.90 ± 163.16 cm; n = 15–20 mice, mean ± SEM, p > 0.05, Dunnett’s test) or appetite (home cage latency to feed: control, 58.50 ± 7.53 s; MIA, 62.42 ± 6.73 s; MIA + exercise, 59.67 ± 6.56 s; n = 12–24 mice, mean ± SEM, p > 0.05, Dunnett’s test) of the mice. Altogether, these results suggest that exercise can attenuate MIA-associated behaviors even in adulthood.

**Exercise in Adulthood Ameliorates Synaptic Defects in MIA Offspring**

Immunostaining of the immediate-early gene c-Fos demonstrated that the running exercise preferentially activated the dentate granule cells compared to its effect on other brain regions (Figures 1D and 1E), in agreement with a previous report (Clark et al., 2011). Therefore, we focused on the properties of the synapses between the granule cell axons, i.e., the mossy fibers, and the CA3 pyramidal cells. First, we measured the developmental changes in the density of mossy fiber synapses, which were defined as puncta that colocalized presynaptic elements (synaptoporin [SPO], a mossy fiber-specific presynaptic marker) and postsynaptic elements (postsynaptic density 95 [PSD95]) in the CA3 (Figures 2A and 2B; Figure S1). The density of mossy fiber synapses decreased from P15 to

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**Figure 1. Adult Exercise Ameliorates MIA-Associated Behaviors**

(A) Social interaction behavior assessed by the three-chamber test and graphed as a social preference index (percentage of total investigation time spent interacting with a stranger), n = 40–45 mice.

(B) Repetitive behavior assessed by self-grooming time. n = 31–33 mice.

(C) Anxiety behavior assessed by the novelty-suppressed feeding test. Latency to feed was used as an index of anxiety and is graphed as the fraction of mice that did not eat over a period of 5 min. n = 12–24 mice.

(D) Representative confocal images of the dentate gyrus and hippocampal CA3 immunostained for NeuN and c-Fos at P60. Mice underwent exercise from P30 to P60. DG, dentate gyrus.

(E) Density (percentage of control) of c-Fos(+) cells in several brain regions in P60 mice that underwent exercise. n = 4 mice (2–6 regions/mouse). S1, primary somatosensory cortex; PRh, perirhinal cortex.

Mean ± SEM (A, B, and E). *p < 0.05 and **p < 0.01 (A–C), and **p < 0.01 versus control (E); Dunnett’s test (A and B), Gehan-Breslow test (C), and Student’s t test (E). Mice from 7 litters in (A), 6 litters in (B), and 4 litters in (C) were used for each condition (control, MIA, and MIA + exercise).
Figure 2. Adult Exercise Ameliorates Synaptic Defects in MIA Offspring

(A) P30 hippocampus immunostained for SPO and PSD95.
(B) Upper: representative images of the mossy fiber pathway stratum lucidum (SL) and CA3 stratum pyramidale (SP) at P30. Lower: colocalized signals of SPO and PSD95 in the boxed areas in the upper images.
(C) Developmental changes in the mossy fiber synapse density. n = 4–9 mice (4–12 regions/mouse).
(D) Mossy fiber synapse density at P60. n = 4–9 mice (10–12 regions/mouse).
(E) mEPSC traces taken from CA3 pyramidal cells from P30–P34 control or MIA offspring.

(legend continued on next page)
P30, probably via developmental synapse elimination, and the decreased synapse density was maintained until P60 in control mice. However, the developmental decrease in synapses was not observed in MIA offspring (Figure 2C), and the synapse density at P15 was maintained until P60 (Figure 2D). The hippocampal neuron density was comparable between control and MIA offspring (granule cell: control, 14.78 ± 0.34 × 10^3/mm^3; MIA, 14.75 ± 0.39 × 10^3/mm^3; pyramidal cell: control, 5.63 ± 0.08 × 10^3/mm^3; MIA, 5.77 ± 0.06 × 10^3/mm^3; mean ± SEM, n = 4 mice per condition, p > 0.05, Student’s t test) and thus was unlikely to contribute to the increased synapse density in MIA offspring. The density of inhibitory synapses was not affected in MIA offspring (Figure S2). Furthermore, we examined the functional properties of the mossy fiber synapses using whole-cell patch-clamp recordings from CA3 pyramidal neurons. We measured the miniature excitatory postsynaptic currents (mEPSCs) derived from the mossy fiber synapses, defined as the difference in the mEPSCs before and those after application of 10 μM DCG-IV, a mGluR2 agonist that specifically blocks mossy fiber transmission (Mellor and Nicoll, 2001). We found that the mEPSC frequency, but not the amplitude, was higher in MIA offspring than in control mice (Figures 2E–2I). Finally, we investigated the structural properties of the large mossy fiber boutons, which include multiple synaptic sites (Rollenhagen et al., 2007), using Thy1-mGFP mice in which the membrane structure of a portion of granule cells was labeled with GFP (Figure 2K) (Tao et al., 2016). We found that the area of each mossy fiber bouton was greater in the MIA offspring than in the controls (Figure 2L), but the overall density of mossy fiber boutons was not changed (Figure 2M). Because the area of mossy fiber boutons and that of postsynapses in the boutons were positively correlated (Figure 2N), these results indicate that the density of functional mossy fiber synapses was increased in adult MIA offspring.

**Exercise in Adulthood Stimulates Microglia to Prune Synapses in MIA Offspring**

Next, we examined whether and how adult exercise affected the synaptic properties of MIA offspring. First, we found that the P30–P60 exercise normalized the increased density of mossy fiber synapses in MIA offspring to the control level (Figure 2C). Moreover, the elevated frequency of mEPSC was reversed by exercise to the control level (Figure 2J). These results suggested that exercise attenuates structural and functional abnormalities of mossy fiber synapses. Next, we examined whether microglia, the brain immune cells that prune synapses during development (Schafer et al., 2012; Paolicelli et al., 2011), were involved in the elimination of mossy fiber synapses. For this purpose, we performed a synapse engulfment assay (Schafer et al., 2012) in the mossy fiber pathway to detect the volume of synaptic elements engulfed by microglia in mice, in which microglia are selectively labeled with GFP (Zhan et al., 2014) (Figure 3A). We confirmed that no GFP(+) cell in the hippocampal CA3 was immunopositive for CCR2 (Prinz and Priller., 2010), an infiltrating cell-specific marker (Figure S3), while GFP(+) cells were stained with an antibody for Siglec-H (Zhang et al., 2006; Konishi et al., 2017) and P2Y12R (Sasaki et al., 2003; Haynes et al., 2006; Mildner et al., 2017), which were generally used as microglia-specific markers (Figure S3). Furthermore, GFP(+) cells expressed interleukin-1β (IL-1β), one of the typical cytokines released from microglia (Figure S3). We also confirmed that the expression patterns of these markers were unchanged, even in MIA or minocycline-injected animals (data not shown). Daily injection of minocycline from P15 (75 mg/kg subcutaneously [s.c.], which is frequently used to suppress microglial activity (Kobayashi et al., 2013), decreased the volume of CD68-positive lysosomes in microglia at P18 (Figures S4A and S4B) and suppressed the developmental decrease in synapse density at P20 (Figure 3B).

Furthermore, PS905 was engulfed by microglia in the mossy fiber pathway (Figure 3A; Figures S4C and S4D), and the engulfment of SPO and the density of microglia were not affected by minocycline treatment (Figures S4E–S4G). These results suggest that microglia selectively engulfed the postsynaptic sites of mossy fiber synapses during development. Whether presynapses or postsynapses are engulfed may depend on the developmental changes of synaptic structure in mossy fiber boutons that occur in the course of synaptic formation (Figure S8A). In P18 MIA offspring, the volume of CD68 (Figure 3C) and that of engulfed PS905 puncta (Figure 3D), but not the density of microglia (Figure 3E), were lower than in control mice, suggesting that the impaired synaptic engulfment by microglia underlies the increased density of mossy fiber synapses in MIA offspring. Finally, we examined whether microglia were involved in the exercise-induced enhancement of synapse elimination in MIA offspring (Figure 2C). To test this idea, we gave MIA offspring daily injections of minocycline throughout the exercise period (P30 to P60). The engulfment of the presynaptic protein VGLUT1 by microglia was lower in the mossy fiber pathway of the MIA offspring than in that of the control mice (Figure 3F). In contrast, exercise promoted synaptic engulfment in MIA offspring to a level comparable to that in control (Figure 3F) without affecting the density of microglia or the CD68 volume (microglial density: control, 1.40 × 10^3 ± 1.7 × 10^3/mm^3; MIA, 1.39 × 10^3 ± 0.15 × 10^3/mm^3; MIA + exercise, 1.59 × 10^3 ± 0.15 × 10^3/mm^3; MIA + exercise + minocycline, 1.42 × 10^3 ± 0.17 × 10^3/mm^3; CD68 of microglia volume: control, 2820 Cell Reports 27, 2817–2825, June 4, 2019
1.62% ± 0.27%; MIA, 2.37% ± 0.19%; MIA + exercise, 2.41% ± 0.60%; MIA + exercise + minocycline, 1.51% ± 0.36%; mean ± SEM, n = 7–8 mice, p > 0.05, Dunnett’s test). In addition, the effect of exercise on synaptic engulfment (Figure 3F) was attenuated by minocycline. In agreement with these results, minocycline treatment resulted in an increase in synapse density (Figure 3G). Because minocycline has various biological actions, we examined whether minocycline directly affects exercise-induced activity in the granule cells. We found that minocycline did not affect the granule cell activity (Figure S5), supporting a more specific action of minocycline on microglia downstream of neural activity, though other indirect effects of minocycline cannot be excluded. For example, minocycline given systemically might affect other cells beyond microglia, including reduction of infiltrating cells. In the current experimental conditions, however, we did not detect evidence of contamination of the
infiltrating cells (Figure S3) in both MIA and minocycline-injected conditions. In addition, the density of Iba1(+) cells was not changed in MIA and minocycline-injected conditions (control, $1.40 \pm 0.17 \times 10^4$ cells/mm$^3$; MIA, $1.39 \pm 0.15 \times 10^4$ cells/mm$^3$; MIA + exercise, $1.59 \pm 0.15 \times 10^4$ cells/mm$^3$; MIA + exercise + minocycline, $1.42 \pm 0.17 \times 10^4$ cells/mm$^3$; mean $\pm$ SEM, $n = 7–8$ mice, $p > 0.05$, Dunnett’s test). From these findings, we speculate that it is likely that minocycline did not affect the number of infiltrating cells under the conditions and regions of interest used in the current study. Altogether, these results suggest that exercise activates microglia in adult MIA offspring to engulf surplus synapses in the mossy fiber pathway.

Neuronal Activation Enhances Microglial Synapse Engagement

Finally, we examined whether increased activity of granule cells is sufficient to induce engulfment of the mossy fiber synapses by microglia, because activity-dependent synapse competition is a key factor that primes microglia to eat weaker synapses (Schafer et al., 2012). For this purpose, we used the chemogenetic technology designer receptors exclusively activated by designer drugs (DREADD), a method for remote and transient manipulation of the activity of cells that express the designer receptors, namely, mutated human muscarinic receptors (hM3Dq and hM4Di) that are exclusively activated by the designer drug clozapine N-oxide (CNO) (Alexander et al., 2009). We first confirmed that the local injection of the adeno-associated virus (AAV) into the dentate gyrus (Figure 4A) successfully labeled the SPO-positive mossy fiber synapses using AAV8-CaMKIIa-eGFP (Figure 4B). Then, we transfected the granule cells with hM3Dq in combination with mCherry (27.0% $\pm$ 3.7% of granule cells expressed mCherry, mean $\pm$ SEM, $n = 6$ mice) to induce neural activity using AAV8-CaMKIIa-hM3Dq-mCherry and confirmed with c-Fos immunoreactivity that hM3D-expressing granule cells increased activity in response to CNO injection (Figure S6).

To examine the interaction between the mossy fiber bouton and the microglia (Figure 4C), we assessed the rate of microglial processes touching hM3Dq-expressing mossy fiber boutons (Figure 4D) and found that the intraperitoneal injection of CNO at P30 decreased the touching events in 24 h (Figure 4E). We
further found that the microglia engulfed more VGLUT1 puncta when the total volume of hM3Dq-expressing boutons was higher in CNO-injected mice (Figure 4F). However, we found no correlation between the engulfment of VGLUT1 puncta and the total volume of hM3Dq-expressing boutons in saline-injected mice (Figure 4F). These results suggest that CNO injection induced activity-dependent synapse competition between hM3Dq-expressing and hM3Dq-non-expressing mossy fiber boutons, resulting in the engulfment of weaker synapses, i.e., hM3Dq-non-expressing synapses, by microglia. In addition, we pharmacologically assessed the role of neuronal activity in the pruning of mossy fiber synapses by using hippocampal slice cultures (Figure S7) (Kasahara et al., 2016). We confirmed that neuronal activity was necessary in the developmental elimination of synapses by microglia and sufficient to induce the elimination of synapses by microglia in slice cultures prepared from MIA offspring (Figures S7C–S7E). Overall, these results suggest that induced competition between the granule cells results in the engulfment of mossy fiber synapses by microglia (Figure S8B).

DISCUSSION

The present study unveiled the role of microglia in the synapse surplus and ASD-related behaviors observed in MIA offspring and its modification by adult voluntary exercise. The modification of dentate gyrus-hippocampal CA3 connections may affect the functional connectivity of the hippocampus with other brain regions, which is associated with autistic-like behaviors in mice (Zhan et al., 2014). In addition, the increased density of functional mossy fiber synapses in adult MIA offspring is consistent with previous studies that demonstrated increased excitability and spine density of hippocampal neurons in rodent models of ASD (Tyzio et al., 2014; Jawaid et al., 2018).

Though we have not assessed the molecular link that mediates neuronal activity and microglial activation in the present study, possible candidates are molecules involved in the classical complement pathway such as C1q and C3. C1q and C3 have been suggested to tag relatively weak or inactive synapses to be engulfed by microglia in the retino-geniculate connections (Schafer et al., 2012; Stevens et al., 2007). Thus, it is possible that complement molecules also contribute to exercise-induced pruning of mossy fiber synapses, because the phenomenon likely depends on synaptic competition. The competition between mossy fiber synapses might be modulated by other molecules, such as brain-derived neurotrophic factor (BDNF), which is enriched in mossy fiber boutons (Koyama et al., 2004) and upregulated by exercise (Voss et al., 2013). Furthermore, BDNF is released from mossy fiber boutons in response to neuronal activity and induces the maturation of synapses (Yoshii and Constantine-Paton, 2010). Therefore, it is possible that exercise-induced granule cell activation promotes the release of BDNF from mossy fiber boutons and strengthens some synapses, leading to the synapse competition. However, changes in hippocampal BDNF levels have not been reported in mouse models of MIA (Han et al., 2016, 2017) except for a decrease in aged (22-month-old) MIA mice (Giovanoli et al., 2015). In addition to these molecules, newly generated granule cells in adulthood would be a key to induce synaptic competition. Adult neurogenesis is enhanced by voluntary exercise (Voss et al., 2013), and adult-born granule cells exhibit higher excitability than existing mature granule cells (Danielson et al., 2016). It has been revealed that MIA inhibits neurogenesis in dentate gyrus during both postnatal days and adulthood, leading to impaired maturation of newborn granule cells (Zhang and van Praag, 2015). Thus, enhanced neurogenesis by exercise may also contribute to competition between synapses derived from adult-born granule cells and existing mature granule cells.

Our results revealed that exercise reversed the abnormal behaviors and synaptic surplus in adult MIA offspring. We determined that exercise activated a portion of neurons, resulting in the engulfment of excess synapses by microglia, likely primed by synaptic competition. However, whether the CA3 synaptic surplus is the underlying cause of the behavioral abnormalities in MIA offspring remains undetermined.

The abnormal behaviors in patients with neurodevelopmental disorders such as ASDs typically appear by 8 to 10 months of age and have been recognized to be irreversible after that period. Reports have shown that autistic-like behaviors may be ameliorated, but the effect was transient or necessitated genetic modification (Yizhar et al., 2011; Mei et al., 2016). In contrast, our findings propose clinically applicable methods to ameliorate ASD symptoms. Further investigations are needed to better understand the cellular and molecular mechanisms that are involved in not only the development but also the reversible aspects of ASD symptoms.

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.05.015.
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AUTHOR CONTRIBUTIONS

M.A. conducted and analyzed the experimental data and wrote the manuscript. K.S., J.O., and K.M. conducted and analyzed the experiments. K.O. helped with electrophysiological experiments. R.K. designed and planned the project and wrote the manuscript. Y.I. discussed the results and commented on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES


# STAR METHODS

## KEY RESOURCES TABLE

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<td>Stock No. 005582</td>
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<tr>
<td>Thy1-mGFP</td>
<td>Dr. Pico Caroni</td>
<td>N/A</td>
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<td><strong>Software and Algorithms</strong></td>
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<td>ImageJ</td>
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<td>MATLAB</td>
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## CONTACT FOR REAGENT AND RESOURCE SHARING

Requests for resources, reagents, or questions about methods should be directed to and will be fulfilled by the Lead Contact, Ryuta Koyama (rkoyama@mol.f.u-tokyo.ac.jp).
**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Animals**
C57BL/6J mice (SLC, Shizuoka, Japan) and mice from the reporter line Thy1-mGFP (Lsi1; a generous gift from Dr. Pico Caroni) and CX3CR1-GFP (Stock No: 005582, The Jackson Laboratory) were maintained under conditions of controlled temperature and a light schedule and provided with unlimited food and water. Pregnant mice were used from E12.5 to make the maternal immune activation (MIA) model (please see Gestational exposure to poly(I:C) in Method Details). All the born pups were used and sacrificed by P67. All experimental procedures conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and to the guidelines provided by the University of Tokyo (approval number: P29-15). Unless otherwise noted, males were used.

**Organotypic culture of hippocampal slices**
Hippocampal slice cultures were prepared from P10 mice (males and females) as previously described with minor modifications (Kasahara et al., 2016). Briefly, the posterior part of the mouse brain was cut into 400-μm thick transverse slices using a DTK-1500 vibratome (Dosaka, Kyoto, Japan) in aerated, ice-cold GBSS (consisting of the following (g/l): 7.00 NaCl, 0.212 MgCl2·6H2O, 2.27 NaHCO3, 0.14 MgSO4·7H2O, 0.286 Na2HPO4·12H2O, 0.37 KCl, 0.22 CaCl2·2H2O, 0.33 KH2PO4, containing 25 mM glucose. The slices were incubated for 30-90 minutes at 4°C with cold incubation medium containing minimum essential medium (MEM) and Hanks’ balanced salt solution (HBSS) (consisting of the following (g/l): 8.00 NaCl, 0.10 MgCl2·6H2O, 0.35 NaHCO3, 0.10 MgSO4·7H2O, 0.12 Na2HPO4·12H2O, 0.40 KCl, 0.185 CaCl2·2H2O, 0.06 KH2PO4) at a ratio of 2:1, 10 mM Tris and 25 mM HEPES. The slices were placed on Omnipore membrane filters (JHWP02500; Millipore) in a solution containing 50% MEM, 25% horse serum (Cell Culture Lab, Cleveland, OH, USA) and 25% HBSS, 10 mM Tris, 25 mM HEPES and 5 mM NaHCO3, supplemented with 33 mM glucose. The slices were then incubated at 37°C in a humidified incubator with 5% CO2 and 95% air. Hippocampal slices were cultured with medium containing 1 mM tetrodotoxin (TTX; Tocris Bioscience, Bristol, UK) or 50 μM picrotoxin (PIC; Sigma) from 5 days in vitro (DIV) to 10 DIV.

**METHOD DETAILS**

**Gestational exposure to poly(I:C)**
The maternal immune activation (MIA) model was made by injecting polyinosinic:polycytidylic acid (poly(I:C) (Potassium salt; Sigma) as previously described (Malkova et al., 2012; Naviaux et al., 2013). Pregnant dams received intraperitoneal (i.p.) injection of poly(I:C) at two doses (3 mg/kg on E12.5 and 1.5 mg/kg on E17.5). The same volume of saline was injected into pregnant dams at the same time to prepare the control mice. Poly(I:C)-injected offspring were used as MIA offspring and weaned at P21. All the born pups were alive and normally developed, though some pregnant mice had abortion (the ratio was 11% for Saline group and 33% for MIA group). No mice were housed alone. Male mice were used for the experiments.

**Running**
Mice in the exercise group were housed in a cage (3–4 mice/cage) with a freely accessible running wheel (130 mm in diameter). The running wheel was placed in the corner of the cage for 30 days from P30. Minocycline (Sigma, 30 mg/kg) was intraperitoneally injected once a day beginning on the first day that the running wheel was placed in the cage. The same volume of saline was injected as a control.

**Sample preparation and immunohistochemistry**
Experimental mice were deeply anesthetized with isoflurane and perfused transcardially with cold phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA). The brain samples were postfixed with 4% PFA for 2–4 h on ice and subsequently immersed in 20% and 30% sucrose in PBS for 24 h and 48 h, respectively, at 4°C. Coronal hippocampal sections (40 μm thick) were prepared with a cryostat (HM520; Thermo Fisher Scientific, Waltham, MA, USA) at −24°C. For slice cultures, samples were fixed overnight in 4% PFA at 4°C. Floating sections were used for PSD95 staining. The fixed samples were rinsed three times with 0.1 M phosphate buffer (PB). Slices were then permeabilized and blocked for 30 min at room temperature in 0.1 M PB with 0.3% Triton X-100 and 10% goat serum. The samples were subsequently incubated with primary antibodies in 0.1 M PB with 0.3% Triton X-100 and 10% goat serum overnight at 4°C. After the samples were rinsed three times with 0.1 M PB, they were incubated with secondary antibodies in 0.1 M PB with 0.3% Triton X-100 and 10% goat serum for 4 h at room temperature. For slice cultures, the samples were incubated with primary antibodies 2 nights at 4°C under agitation and secondary antibodies and NeuroTrace (435/455 blue fluorescent Nissl stain, 1:200; Thermo Scientific, MA, USA) 1 overnight at 4°C under agitation. Finally, the samples were rinsed three times with 0.1 M PB and embedded in VECTASHIELD with DAPI (Vector Laboratories, Burlingame, CA, USA) or MOUNTANT (Thermo Scientific). The primary antibodies used in this study were as follows: rabbit anti-SPO (1:1000; Synaptic Systems, Göttingen, Germany), mouse anti-PSD95 (1:500; Thermo Scientific), guinea pig anti-VGLUT1 (1:1000; Synaptic Systems), mouse anti-VGAT (1:1000; Synaptic Systems), rabbit anti-gephyrin (1:1000; Synaptic Systems), rabbit anti-Iba1 (1:400; Wako, Osaka, Japan), rat anti-CD68 (1:200; Bio-Rad, CA, USA), chicken anti-GFP (1:1000; Abcam, Cambridge, UK), mouse anti-mCherry (1:1000; Abcam), guinea pig anti-Homer1 (1:500, Synaptic Systems), rabbit anti-DsRed (1:1000, Takara Bio, Open Access Cell Reports 27, 2817–2825.e1–e5, June 4, 2019 e2
Shiga, Japan), rabbit anti-CCR2 (1:100, Abcam), rabbit anti-P2Y12 (1:500, AnaSpec, CA, USA) and mouse IL-1β (1:100, Cell Signaling Technology, MA, USA). Secondary antibodies conjugated to Alexa fluor dyes (1:500; Invitrogen, MD, USA) were used.

c-Fos immunostaining was performed on free-floating sections as described below. The samples were incubated for 1 h in 0.2% Triton X-100 in PBS containing 5% goat serum and with rabbit anti-c-Fos (1:1000; Santa Cruz Biotechnology, CA, USA) and mouse anti-NeuN (1:1000; Millipore, Bedford, MA, USA) overnight at 4°C. The sections were then incubated with the goat anti-rabbit biotinylated secondary antibody (1:500; Vector Laboratories) and Alexa fluor dye-conjugated secondary antibodies (1:500; Invitrogen) for 4 h at room temperature, with the avidin-biotin complex (1:100; Vector Laboratories) for 1.5 h at room temperature, and with CY3 (1: 1000; PerkinElmer, MA, USA) for 1 h at room temperature. After three rinses, the samples were embedded. Or the samples were incubated for 1 h in 0.3% Triton X-100 in PBS containing 5% bovine serum albumin and with goat anti-c-Fos (1:100; Santa Cruz Biotechnology, CA, USA) and mouse anti-NeuN (1:1000; Millipore, MA, USA) overnight at 4°C. The sections were then incubated with Alexa fluor dye-conjugated secondary antibodies (1:500; Invitrogen) for 1.5 h at room temperature. After three rinses, the samples were encapsulated.

For Siglec-H immunostaining, CX3CR1^{GFP}/+ mice were perfused with cold phosphate-buffered saline (PBS) followed by 2% paraformaldehyde (PFA). The brain samples were immersed in 30% sucrose in PBS for 24 h at 4°C. Coronal hippocampal sections (30 μm thick) were prepared with a cryostat and mounted on glass slides. The samples were incubated for 1 h in 0.3% Triton X-100 in PBS containing 5% bovine serum albumin and with goat anti-GFP, rabbit anti-Iba1 and sheep anti-Siglec-H (1:1000, provided by Dr. Konishi, assistant professor at Nagoya University) overnight at 4°C. The sections were then incubated with Alexa fluor dye-conjugated secondary antibodies (1:500; Invitrogen) for 1 h at room temperature. After three rinses, the samples were encapsulated.

Stereotoxic surgery for virus infection
MIA offspring were used at P10. Mice were anesthetized using pentobarbital (25 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and placed in a stereotoxic apparatus. The virus (0.5 μL per side) was injected into the bilateral dentate gyrus (AP: −1.9 mm, LM: ± 0.9 mm, DV: −1.8 mm) at a rate of 0.25 μL/min using glass pipettes. For the expression of DREADD, we bilaterally injected AAV8-CaMKIIa-eGFP or AAV8-CaMKIIa-hM3Dq-mCherry (purchased from Addgene) using glass pipettes, which were then left in place for a few minutes before they were slowly removed. Then, the mice were intraperitoneally injected with the selective ligand clozapine-N-oxide (CNO; Abcam, 5 mg/ kg) or saline at P30 and sacrificed at P31 for further analysis.

Three-chamber social interaction test
A three-chamber test was carried out to investigate the social interaction of mice. The test was performed in a white three-chamber box (25 × 51 × 25 cm). Age- and gender-matched C57BL/6J mice that had never been exposed to the test mice were used as stranger mice and individually placed in a chamber. In the other side of the chamber, a cage-mate mouse was used as a familiar mouse. Both the stranger and familiar mice were placed in wire cups to allow the test mice to freely access and sniff the mice in the cups. The three-chamber box and cups were cleaned with 70% ethanol and wiped with paper towels between each trial. In the first 10-min session, a test mouse was placed in the three-chamber box, where two empty cups were located in both sides of the chamber, and allowed to freely explore to habituate the test mouse. In the second 10-min session, a stranger mouse and a familiar mouse were placed in the cage on each side. Then, the test mouse was placed in the center chamber of the three-chamber box and allowed to freely explore the box 10 min. The brightness of the room was 10 lux throughout the test. The movement of the mice was recorded by a USB webcam and PC-based video capture software. The recorded video file was further analyzed by ImageJ, and the time spent in each chamber was measured. A preference index to stranger mice was calculated to assess the social interaction using the following formula: 100 × time in stranger chamber/total time in stranger and familiar chamber.

Grooming test
A grooming test was performed the day after the three-chamber test to investigate repetitive behaviors. A test mouse was placed in a normal cage without wood bedding for 20 minutes, in which the first 10 minutes was the habitation session and the last 10 minutes was the test session. The movement of the test mouse was recorded by a USB webcam and PC-based video capture software. The recorded video file was further analyzed to manually measure the grooming time during the test session.

Novelty-suppressed feeding test
The novelty-suppressed feeding test was carried out for 5 min in a white polystyrene box (40 × 40 × 30 cm) whose floor was covered with wood bedding. Twenty-four hours before testing, all food was removed from the home cage, but water was freely accessible during this period. During the test period, a single pellet was placed on a white paper platform located in the center of the test box at which the light intensity was approximately 1000 lux. The test mouse was placed in a corner of the box, and the latency from the starting time until the time when the mouse bit the food pellet in the center of the field was measured to examine the level
of anxiety. Immediately after this test, the mice were transferred to their home cage, and the latency to feed in their home cage was measured to examine their basal appetite.

**Open field test**

The open field test was conducted using a large, square, white polystyrene box (40 × 40 × 30 cm) with an open top and a floor covered with clear acrylic sheeting and lasted for 10 min. The arena of the open field included the center zone (27 × 27 cm). The movement of the test mouse was recorded by a USB webcam and PC-based video capture software, and the recorded video file was analyzed using ImageJ to measure the total distance traveled.

**Electrophysiology**

P30–34 (before exercise) or P60-67 (after exercise) mice were deeply anesthetized with isoflurane, and the brains were quickly removed and placed in ice-cold, oxygenated (95% O2/5% CO2) artificial cerebrospinal fluid (ACSF) containing 127 mM NaCl, 1.6 mM KCl, 1.24 mM KH2PO4, 1.3 mM MgSO4, 2.4 mM CaCl2, 26 mM NaHCO3, and 10 mM glucose. Transverse slices containing the CA3 area were cut at a thickness of 400 μm with a vibratome in ice-cold, oxygenated modified ACSF containing 222.1 mM sucrose, 27 mM NaHCO3, 1.4 mM NaH2PO4, 2.5 mM KCl, 0.5 mM ascorbic acid, 1 mM CaCl2, and 7 mM MgSO4. Slices were maintained for 30 min at 37°C and then incubated for at least 30 min at room temperature before use.

Slices were transferred to a recording chamber and superfused with oxygenated ACSF containing 0.1 mM picrotoxin (30–33°C, 1–3 mL/min). Whole-cell recordings were made from visually identified, pyramidal neurons located in the CA3 region using infrared differential interference contrast (IR/DIC) techniques. Patch pipettes (3-6 MΩ) were fabricated from borosilicate glass and filled with a solution containing 127 mM CsMeSO4, 8 mM CsCl, 10 mM HEPES, 1 mM MgCl2, 10 mM phosphocreatine-Na2, 4 mM MgATP, 0.3 mM NaGTP, and 0.2 mM EGTA (pH 7.2–7.3, 280–295 mOsm). mEPSCs were recorded at a holding potential of −70 mV in the presence of tetrodotoxin (1 μM). mEPSCs were detected using an in-house MATLAB program and were defined as inward currents with amplitudes > 7 pA. DCG-IV (Tocris Bioscience, 10 μM for slices from P30-34 mice and 1 μM for slices from P60-67 mice) was applied to block synaptic transmission from mossy fiber synapses. The series resistance was monitored, and if it exceeded 40 MΩ, the data were discarded. Data were sampled at 20 kHz and filtered at 2 kHz using an Axopatch 200B, 700B amplifier (Molecular Devices), Digidata 1320A, 1440A (Molecular Devices), and pClamp 10.2 (Molecular Devices).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Quantification**

The immunostained samples were analyzed with a TCS SP8 (Leica Microsystems, Wetzlar, Germany) or FV1200 (Olympus, Tokyo, Japan) confocal system under 10 ×, 20 ×, 60 × and 100 × objectives. Z series images were collected with 0.33–μm steps, and 4 Z sections (1 μm thick) were stacked using ImageJ (NIH) to quantify the synapse density. 11 Z series sections (5 μm thick) for the quantification of synapse engulfment or 21 Z series sections (10 μm thick) for CD68 expression were collected at 0.50 μm steps. 25 Z series sections (8 μm thick) for the quantification of post-synaptic volume in mossy fiber bouton were collected at 0.33 μm steps. ImageJ was used to quantify the synapse density. After the stacked images in the stratum lucidum were cropped, pre- and postsynaptic signals were detected by determining the threshold of the fluorescent intensities. Then, the number of colocalized signals of pre- and postsynaptic puncta were counted.

The quantification of synapse engulfment and CD68 expression was carried out as previously described with minor modifications (Schafer et al., 2012). Microlglial, synaptic, and CD68 immunofluorescent signals were detected by determining the threshold of the fluorescent intensities in ImageJ. Then, colocalized images of microglia and synapses or CD68 were prepared, and the volume of microglia and the colocalization were measured in the stratum lucidum. To determine the synapse engulfment by microglia, the following calculation was used: Volume of internalized synaptic puncta (μm³)/ Volume of microglia (μm³). To determine the % of CD68 expression, the following calculation was used: Volume of internalized CD68 (μm³)/ Volume of microglia (μm³).

For assays of microglia-dependent synaptic pruning in hippocampal slices, microglial, synaptic, and CD68 immunofluorescent signals were detected by determining the threshold of the fluorescent intensities in ImageJ. Then, colocalized images of microglia, synapses and CD68 were prepared, and the volume of microglia and the colocalization were measured in the stratum lucidum. To determine the synaptic engulfment by microglia, the following calculation was used: Volume of internalized synaptic puncta (μm³)/ Volume of microglia (μm³).

The quantification of post-synaptic volume in mossy fiber bouton of Thy-1 mGFP mice was carried out as follows; GFP-labeled bouton and postsynaptic immunofluorescent signals were detected by determining the threshold of the fluorescent intensities in ImageJ. Then, colocalized images of boutons and synapses were prepared, and the area of boutons and the colocalization were measured in the stratum lucidum. All analyses were carried out in a blinded manner.

**Statistical Analysis**

The data are represented as the mean ± standard error of the mean (SEM) or as box and whisker plots showing the distribution and median (solid line) of the data (box edges indicate 25th and 75th percentiles; whiskers, min and max) and were pooled from at least 3
independent experiments. The data were collected and statistically analyzed independently by 2 researchers in a blinded manner. Student’s t test or Dunnet’s test, or one-way or two-way analysis of variance (ANOVA) followed by the Tukey test was performed for statistical analysis unless otherwise described. The Gehan-Breslow test (Figure 1C) was also used.

DATA AND SOFTWARE AVAILABILITY

Reasonable requests for data will be fulfilled by the Lead Contact.