Title
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Publication Date
2022

DOI
10.1177/26331055211071124

Peer reviewed

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Neuroscience Insights
Volume 17: 1–4
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DOI: 10.1177/26331055211071124
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ABSTRACT: Microglia are the primary immune cells in CNS. Recent work shows that microglia are also essential for proper brain development through synaptic pruning and remodeling during early life development. But the question of whether and how microglia regulate synaptic connectivity in the adult brain remains open. Our recently published study provides new insights into the functional roles of microglia in the adult mouse brain. We find that chronic depletion of microglia via CSF1R inhibitors in the visual cortex in adult mice induces a dramatic increase in perineuronal nets, and enhances neural activities of both excitatory neurons and parvalbumin interneurons. These findings highlight new potential therapeutic avenues to enhance adult neural plasticity by manipulating microglia.

KEYWORDS: Microglia, excitatory, parvalbumin, visual cortex, plasticity

RECEIVED: November 19, 2021. **ACCEPTED:** December 17, 2021.

TYPE: Commentary

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The author disclosed receipt of the following support by US National Institutes of Health (NIH) grants 1R01NS083801 and RF1AG056768 to KNG, R01EY028212 and RF1AG065675 to XX, and R35GM127102 to TCH.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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COMMENT ON: Yong-Jun Liu, Elizabeth Spangenberg, Bryan Tang, Todd C Holmes, Kim N Green, Xiangmin Xu. Microglia elimination increases neural circuit connectivity and activity in adult mouse cortex. The Journal of Neuroscience. 2021 February; 41(6):1274–1287. doi: 10.1523/JNEUROSCI.2140-20.2020. Epub 2020 Dec 30. PMID: 33380470.

Introduction

Microglia are the primary immune cells in the mammalian CNS. They are involved in pathological processes in our brains, including inflammation, stroke, neurodegenerative diseases, and viral and bacterial infection.[1] Beyond their known neuroimmunological roles, recent studies reveal that microglia functionally contribute to synaptic remodeling and maintenance of synaptic structure during development.[2,3] An earlier technical innovation allowed us to begin to address the function of microglia in the adult brain; pharmacological removal of microglia showed that microglia continue to regulate neuronal structures in the adult brain, and CSF1R signaling is critical during the processing.[4] The administration of CSF1R inhibition elicits a rapid and extensive depletion of microglia in the adult brain. This manipulation is reversible after the inhibitor treatment ceases.[4,5] Pharmacological depletion of microglia induces increased spine density in adult mice and rescues dendritic spine loss and lowers neuronal loss in Alzheimer's model mouse.[6] Similar manipulations prevent the loss of perineuronal nets in mouse models of Huntington's disease.[7] However, how microglia regulates neural connections in adult brains remained unclear. We reported in 2021 that chronic microglia depletion in the adult visual cortex induces increases in neural circuit connectivity, and enhances activity in the adult mouse cortex.[8] Notably, microglia depletion increases the connectivity of both excitatory and inhibitory neurons. Furthermore, these changes reverse after microglia repopulation.[3,4] Our new results reveal a prominent role of microglia in modulating neural plasticity and regulating subsequent functional activity in adult brains. This presents new opportunities to better treat brain disorders disease by manipulating microglia. We summarize and highlight our novel findings below.

Microglia Depletion via CSF1R Inhibitors Enhances PNNs

Administration of colony-stimulating factor 1 receptor (CSF1R) inhibitor PLX3397 (290ppm in Ain76A chow) or PLX5622 for 2 to 3 weeks leads to robust microglia depletion as shown in Figure 1a, the microglia marker IBA1 is reduced after 2 weeks of PLX3397 treatment. And after withdrawal of the inhibitor treatment, the microglia recover to pretreatment levels in 2 weeks (Figure 1a, right panel). Perineuronal nets (PNNs) are primarily composed of chondroitin sulfate proteoglycans of the aggrecan family, as well as hyaluronic acid, and tenascin-R.[9] PNNs are extracellular matrix structures that preferentially surround GABAergic neurons which are known to mediate adult neural plasticity.[3] The vegetable lectin Wisteria floribunda agglutinin (WFA) is used to detect PNNs. PNN density and signal intensity increases following microglia depletion relative to untreated controls (Figure 1b). Microglia

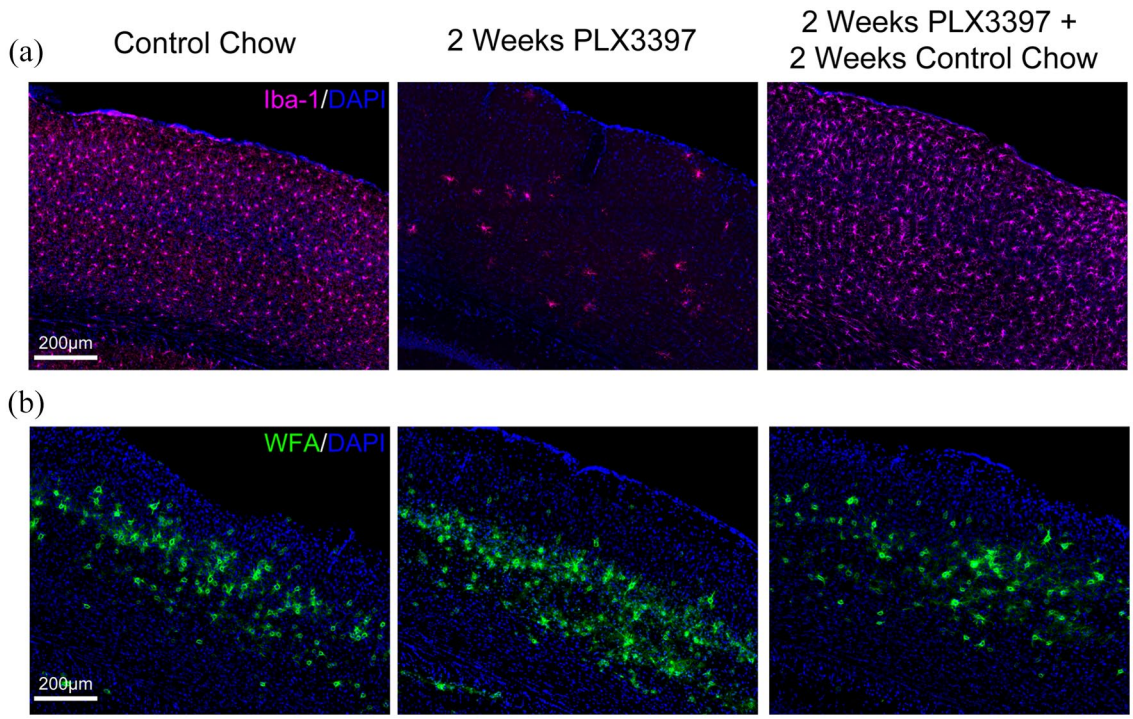

Figure 1. Microglia depletion increases PNNs in the visual cortex following 2-week CSF1R inhibitors PLX3397 treatment: (a) example IBA1 immunostaining of V1 in control mice (left), depletion of microglia in mice treated with PLX3397 (middle) and repopulated microglia during a 2-week recovery period (right) and (b) WFA staining of PNNs (green) alongside DAPI staining (blue) in V1 under control, microglia depletion, and microglia repopulation conditions. These original data are associated with our published study.[8]

depletion also upregulates the expression intensity of parvalbumin (PV) neurons, as measured by anti-PV staining in adult V1 mice.

Presynaptic EPSC Inputs to Pyramidal Neurons in V1 Increases With Microglia Depletion

Whole-cell recordings in V1 excitatory neurons of acute brain slices from control mice and PLX3397 treated mice showed that spontaneous EPSC frequencies increase dramatically following microglia depletion relative to controls. However, the intrinsic membrane properties are not regulated by CSF1R inhibition. We mapped local synaptic inputs to recorded neurons using the laser scanning photostimulation (LSPS) approach. The results demonstrate pyramidal neurons in the PLX3397 treated group have stronger EPSC amplitudes and higher events number (Figure 2a) and show enhanced local excitatory connections compared with control mice (Figure 2b). Remarkably, the mean total ESPCs inputs for adult mice are quadrupled in the absence of microglia (Figure 2d; $P = 1.48 \times 10^{-6}$).; Mann–Whitney U test). Consistently, the numbers of total EPSC inputs per cell also differ with PLX3397 treatment (Figure 2e; $P = 3.97 \times 10^{-7}$, Mann–Whitney U test). Previously we recorded from V1 Layer 5 neurons in younger mice (P21-28) and find that overall excitatory inputs and amplitudes are similar to the PLX3397 treated older mice (Figure 2b and c). These results suggest that microglial elimination restores excitatory connectivity to an earlier developmental stage, thus potentially re-opening adult plasticity.

PV-specific IPSC Inputs to Cortical Pyramidal Neurons Increase With Microglia Depletion

Although the excitatory connections to pyramidal neurons are enhanced after microglia depletion, there are no abnormal behaviors are evoked.[4,5] Furthermore, neuronal spikes do not change substantially compared with control cells mapping by photostimulation. The absence of a hyperexcitable phenotype suggests that corresponding inhibitory inputs must scale to balance the increased excitatory connections following microglia depletion. PV interneurons play important roles in modulating adult cortical excitation.[10,11] PV inhibitory connections to pyramidal neurons were mapped by ChR2 photoactivation of presynaptic PV neurons in PV-Cre; Ai32 mice.[12] The results show that excitatory neurons receive greater inhibitory inputs from PV neurons in the microglia-deleted brains. This supports the idea that microglia modulate both excitatory and inhibitory connections during normal development in V1.

Microglia Removal Enhances Excitatory and Inhibitory Activity In Vivo Awake Adult Mouse Cortex

To investigate the large-scale neural circuit effects in awake adult mice in vivo following microglia removal, we performed two-photon calcium imaging of a large population of neurons in the binocular V1 area longitudinally in awake CaMK2-tTA;

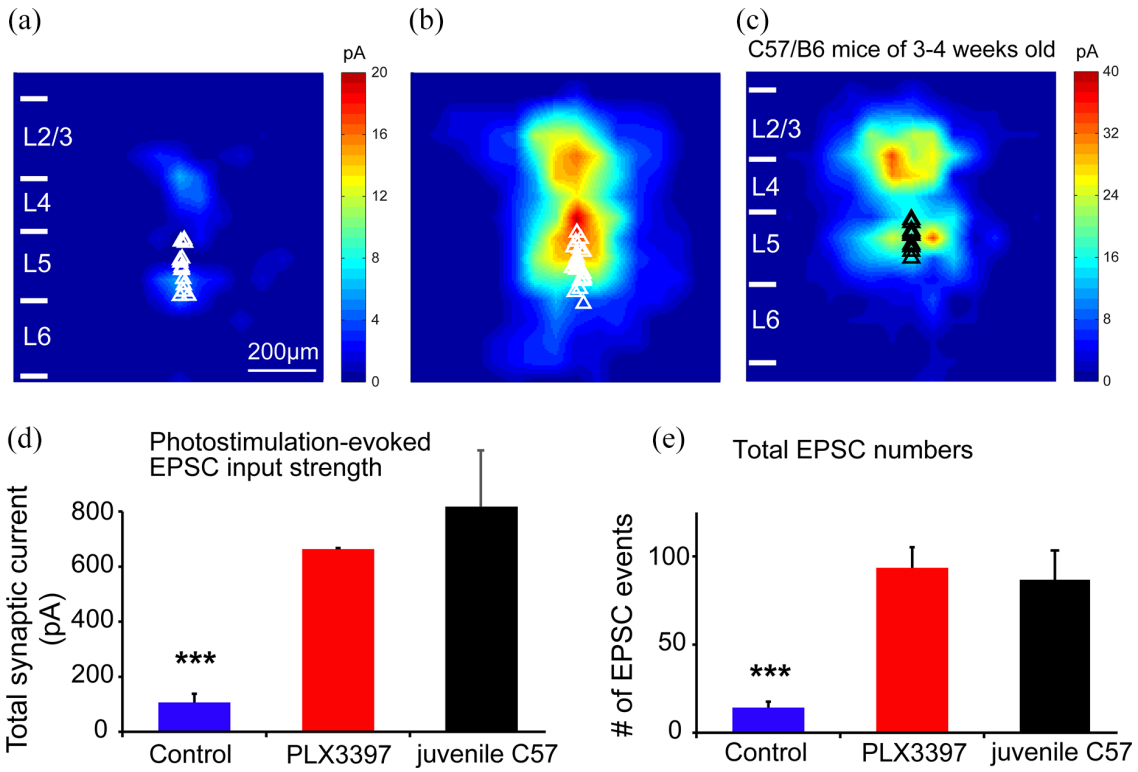

Figure 2. Local excitatory connections to excitatory pyramidal neurons enhanced following 3 weeks PLX3397 treatment. (a) Averaged photostimulation-evoked EPSC input maps of layer 5 pyramidal neurons from adult mouse visual cortex in control mice, and in PLX3397 treated mice (b). (c) overall excitatory inputs and amplitudes recorded from V1 Layer 5 pyramidal neurons in younger mice (P21-28) were similar to the PLX3397 treated mice. (d) Average strengths of summed EPSC inputs and (e), total EPSC numbers in PLX3397 treated groups increase significantly compared with control cells in adult mice. Microglial elimination restores excitatory connectivity to that of juvenile mice. These original data are associated with our published study.[8] ***$P < .001$.

tetO-GCaMP6s mice expressing GCaMP6s in excitatory neurons,[13] and in PV-Cre; Ai163 mice which have GCaMP6s selectively expressed in inhibitory PV neurons. Baseline pre-treatment recordings were collected, then mice were treated with ~3 weeks of food treated with either PLX5622 or PLX3397 to remove microglia. Controls received no drug and continued to eat their normal diet. The neural activities of layer 2/3 pyramidal neurons or PV neurons in V1 were recorded every 4 days.

Two-photon calcium imaging reveals that microglia depletion induces sustained increases of excitatory neuron activities and PV$^+$ inhibitory neuron activities in the adult mouse visual cortex compared with control mice (overall $P = .001$, Kruskal–Wallis test). The results are consistent with slice-based circuit mapping data following CSF1R inhibitor treatment, and confirm that microglia regulate the activities of both excitatory neurons and PV inhibitory neurons in the adult cortex *in vivo*.

Conclusions

Our new data establish that microglial depletion effectively regulates adult cortical connectivity and functional neural activity in addition to their known regulatory role during the early postnatal development. This is supported by other recent studies of eliminating microglia from developmental and adult mouse brains.[14] Acute microglial depletion in adult mice via targeted diphtheria toxin leads to changes in dendritic spine dynamics.[15] Our prior studies show that chronic microglia depletion by CSF1R inhibitors in adult mice leads to robust increases in dendritic spine densities,[4,16,17] as well as increases in synaptic puncta. Furthermore, young mice deficient for C1q or the downstream complement protein C3 exhibit excess synapses as a result of a pruning failure.[18,19] Microglia depletion by CSF1R inhibitor PLX5622 during the first 2 postnatal weeks, PV inhibitory synapses onto excitatory neurons were increased in mouse S1 cortex. Furthermore, microglia expressing the GABAB1 receptor (GABAB1R) selectively sculpt cortical inhibitory circuits without impacting excitatory synapses during neural development. Ablation of GABAB receptors within microglia disrupts this process and produces behavioral abnormalities.[20] Using an optogenetic approach, we show that following microglia depletion, PV+ inhibitory synaptic connections to excitatory neurons are increased. This demonstration establishes that microglia can sculpt both excitatory and inhibitory synaptic connections in cortical circuits in adults. These changes of increased circuit connectivity now include robust changes in perineuronal nets following microglial depletion. In conclusion, our study establishes that microglia regulate neural circuit connectivity and activity by modifying both excitatory and inhibitory synaptic connections to excitatory neurons in the

adult cortex. These results reveal new potential avenues to enhance adult neural plasticity, which can be applied to treat brain disorders and injuries including both neurodegenerative diseases and neuropsychiatric disorders.

Author Contributions

Yong-Jun Liu, Todd Holmes, Kim N Green, and Xiangmin Xu wrote, edited and commented on the manuscript. Xiangmin Xu conceived and oversaw this writing project.

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