Neuronal gamma oscillations and activity-dependent potassium transients remain regular after depletion of microglia in postnatal cortex tissue

Andrea Lewen | Thuy-Truc Ta | Tiziana Cesetti | Jan-Oliver Hollnagel | Ismini E. Papageorgiou | Bruno Chausse | Oliver Kann

Abstract
Microglial cells (resident macrophages) feature rapid activation in CNS disease and can acquire multiple phenotypes exerting neuroprotection or neurotoxicity. The functional impact of surveying (“resting”) microglia on neural excitability and neurotransmission in physiology is widely unknown, however. We addressed this issue in male rat hippocampal slice cultures (in situ) by pharmacological microglial ablation within days and by characterizing neuronal gamma-band oscillations (30–70 Hz) that are highly sensitive to neuromodulators and disturbances in ion and energy regulation. Gamma oscillations support action potential timing and synaptic plasticity, associate with higher brain functions like perception and memory, and require precise communication between excitatory pyramidal cells and inhibitory (GABAergic) interneurons. The slice cultures featured well-preserved hippocampal cytoarchitecture and parvalbumin-positive interneuron networks, microglia with ramified morphology, and low basal levels of IL-6, TNF-α, and nitric oxide (NO). Stimulation of slice cultures with the pro-inflammatory cytokine IFN-γ or bacterial LPS serving as positive controls for microglial reactivity induced MHC-II expression and increased cytokine and NO release. Chronic exposure of slice cultures to liposome-encapsulated clodronate reduced the microglial cell population by about 96%, whereas neuronal structures, astrocyte GFAP expression, and basal levels of cytokines and NO were unchanged. Notably, the properties of gamma oscillations reflecting frequency, number and synchronization of synapse activity were regular after microglial depletion. Also, electrical stimulus-induced transients of the extracellular potassium concentration ([K+]o) reflecting cellular K+ efflux, clearance and buffering were unchanged. This suggests that nonreactive microglia are dispensable for neuronal homeostasis and neuromodulation underlying network signaling and rhythm generation in cortical tissue.
1 | INTRODUCTION

Microglia are the tissue-resident macrophages that become activated in most CNS diseases, such as stroke, bacterial meningoencephalitis, multiple sclerosis, and Alzheimer’s disease (Colonna & Butovsky, 2017; Prinz, Jung, & Priller, 2019). The activation of microglia under such pathophysiological conditions is complex and associates with morphological changes, proliferation, antigen presentation, release of cytokines and free radicals, migration, and phagocytosis; the functional consequences range from neuroprotective to neurotoxic (Deczkowska, Amit, & Schwartz, 2018; Hanisch & Kettenmann, 2007; Ransohoff & Perry, 2009).

To sense danger signals and/or homeostatic imbalance within the brain parenchyma, microglia express a variety of receptors that recognize, for example, bacterial and viral components, modified endogenous ligands, neurotransmitters, cytokines, and chemokines (Deczkowska et al., 2018; Kettenmann, Kirchhoff, & Butovsky, 2017; Prinz et al., 2019). The activation of microglia during normal brain information processing is widely unknown, however. We show that neuronal gamma oscillations (30–70 Hz) reflecting precise communication between different types of neurons as well as extracellular potassium ion dynamics reflecting the activities of neurons and astrocytes remain regular after pharmacological removal of microglia from postnatal cortex tissue. Our data support the biological concept that nonreactive microglia are dispensable for neuronal homeostasis, signaling, and rhythm generation in the healthy postnatal brain.

Microglia are resident immune cells that become reactive in most brain diseases, such as stroke, bacterial meningoencephalitis, and Alzheimer’s disease. The role of nonreactive (surveying) microglia during normal brain information processing is widely unknown, however. We show that neuronal gamma oscillations (30–70 Hz) reflecting precise communication between different types of neurons as well as extracellular potassium ion dynamics reflecting the activities of neurons and astrocytes remain regular after pharmacological removal of microglia from postnatal cortex tissue. Our data support the biological concept that nonreactive microglia are dispensable for neuronal homeostasis, signaling, and rhythm generation in the healthy postnatal brain.
2 | MATERIALS AND METHODS

2.1 | Animals

Wistar rats were purchased from Charles River (Sulzfeld, Germany) and handled in accordance with the European directive 2010/63/EU and with the consent of the animal welfare officers at the University of Heidelberg (licenses, T56/11, T46/14, and T96/15). Experiments were performed and reported in accordance with the ARRIVE guidelines.

2.2 | Preparation and exposures of slice cultures

Organotypic hippocampal slice cultures were prepared as described (Kann et al., 2003; Papageorgiou et al., 2016). In brief, hippocampal slices (400 μm) were cut with a McIlwain tissue chopper (Mickle Laboratory Engineering Company Ltd., Guildford, UK) from 49 male rats at postnatal day 9 or 10 (p9-p10) under sterile conditions. Male rats were used to avoid the effects of sex-related variability in microglial and neuronal biology (Bordt, Ceasrine, & Bilbo, 2020). Slices with incomplete hippocampal structures were rejected. Three to five slices were maintained on Biopore™ membranes (Millicell standing inserts, Merck Millipore, Darmstadt, Germany), at the interface between serum-containing culture medium and humidified normal atmosphere enriched with 5% CO2 (36.5°C) in an incubator (Heracell, Thermoscientific, Dreieich, Germany). The culture medium consisted of 50% minimal essential medium, 25% Hank's balanced salt solution (Sigma-Aldrich, Taufkirchen, Germany), 25% heat-inactivated horse serum (Life Technologies, Darmstadt, Germany), and 2 mM L-glutamine (Life Technologies) at pH 7.3 titrated with Trisbase. The culture medium (1 ml) was replaced three times per week.

From each preparation, membranes with slice cultures were randomly assigned to experimental groups. Slice cultures were used for electrophysiological recordings, fixed for immunohistochemistry or toluidine staining. For biochemical analysis, the “conditioned” culture medium was collected after 24 hr or 72 hr and stored at −80°C. The liposome-containing medium was centrifuged.

Slice cultures were exposed for 7 to 12 days in-vitro (DIV) to liposome-encapsulated clodronate (Liposoma B.V., Amsterdam, The Netherlands) to deplete the microglial cell population (Vinet et al., 2012). Liposomal clodronate was continuously present in the culture medium at a final concentration of 100 μg/ml from DIV 0 onward (Papageorgiou et al., 2016; Ta et al., 2019).

Slice cultures were stimulated by exposures to the pro-inflammatory cytokine IFN-γ or to bacterial lipopolysaccharide (LPS). The stock solution of IFN-γ was prepared in 10 mM sterile-buffered sodium phosphate and further diluted in the culture medium to final concentrations of 100 ng/ml or 1,000 ng/ml. LPS (from Escherichia coli, serotype R515 (Rel) was ready-to-use and further diluted in the culture medium to the final concentration of 100 ng/ml. Aliquots of solutions were kept at −20°C. IFN-γ was purchased from PeproTech GmbH (Hamburg, Germany); LPS was purchased from Alexis Biochemicals (Enzo Life Sciences GmbH, Lorrach, Germany).

2.3 | Biochemical analysis of the culture medium

All enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D (R&D Systems, Inc., Minneapolis, MN, USA) and applied according to the supplier’s protocol for the detection of interleukin 6 (IL-6; Cat. num. DY506) and tumor necrosis factor-alpha (TNF-α; Cat. num. DY510). Concentrations of antibodies strictly followed the suppliers’ protocol. Wash buffer consisted of 0.05% Tween 20 (Merck-Millipore; Darmstadt, Germany) in PBS. Capture antibodies were diluted in PBS (pH 7.2–7.4) and the reaction plate was coated overnight. The detection antibody for TNF-α was diluted in the reagent diluent, consisting of 1% bovine serum albumin in PBS (pH 7.2–7.4); the detection antibody for IL-6 was diluted in 2% normal goat serum in reagent diluent. Ten-point standard curves were constructed from nine sequential twofold dilution steps of recombinant IL-6 (8,000 pg/ml) or TNF-α (4,000 pg/ml), and a negative control containing only reagent diluent. Samples were incubated in the coated reaction plate for 2 hr. The detection antibody was then applied for 2 hr and visualized with tetramethylbenzidine substrate solution (Moss Inc., Pasadena, USA). The development reaction was stopped with sulfuric acid, and the optical density was determined with a microplate reader (iMark Microplate Absorbance Reader, Bio-Rad Laboratories GmbH, Munich, Germany) at 450 nm (with 540 nm reference). The concentrations of TNF-α and IL-6 (both pg/ml) were estimated by using the quadratic fit.

Nitric oxide (NO) release was quantified by determining the levels of the stable metabolite nitrite using a Griess reaction-based assay that was carried out with undiluted culture medium. Nine-point standard curves were constructed by twofold dilution steps of an 80 μM sodium nitrite high standard (Merck Chemicals, Darmstadt, Germany). After the addition of the Griess reagent mixture (0.05% 1-naphthylethylenediamine hydrochloride, 0.5% sulfanilamide and 2.5% orthophosphoric acid), the optical density was measured with a microplate reader at 540 nm (Bio-Rad). The molarity of NO (μM) was calculated from the standard curve using a linear fit.

2.4 | Immunohistochemistry and toluidine blue staining of slice cultures

Slice cultures were fixed for at least 2 hr with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 6.8), incubated for 2–3 hr in 30% sucrose (AppliChem GmbH, Darmstadt, Germany) and cut into 25-μm sections with a cryostat (CM1850; Leica Biosystems, Nussloch, Germany). Immunohistochemistry was conducted in free-floating sections. All primary antibodies were diluted in PBS + 0.3% Triton™ X-100 + 10% normal goat serum (Life Technologies). Secondary antibodies were diluted in 0.2% bovine serum albumin dissolved in PBS + 0.3% Triton™ X-100. Several washing steps with
PBS were conducted, for example, after blocking of unspecific binding sites or antibody applications.

For immunohistochemistry of ionized calcium-binding adapter molecule 1 (Iba1), parvalbumin-positive interneurons, glial fibrillary acidic protein (GFAP), and MHC class II (MHC-II) (see Table 1) unspecific immunoglobulin reactions were blocked for 1 hr with 10% normal serum. Primary antibodies were rabbit polyclonal anti-Iba1 (Fujifilm-WAKO Chemicals Europe GmbH, Neuss, Germany, RRID:AB_839504), mouse anti-PV (Sigma-Aldrich, RRID:AB_477329), rabbit polyclonal anti-GFAP (Agilent-Dako Denmark A/S, Glostrup, Denmark, RRID:AB_10013382) all diluted 1:1,000. Mouse monoclonal anti-MHC-II was diluted 1:250 (Abcam, Cambridge, UK, RRID:AB_447796). Cryosections were exposed overnight to the primary antibody. Unspecific binding sites of the secondary antibodies were blocked for 1 hr in 0.2% bovine serum albumin (Carl Roth GmbH & Co. KG, Karlsruhe, Germany). Secondary antibodies used were as follows: for Iba1 and GFAP, biotin-conjugated goat anti-rabbit (Vector Laboratories Inc., CA, USA) diluted 1:1,000; for PV and MHC-II, biotin-conjugated horse anti-mouse (Vector Laboratories) diluted 1:1,000. The secondary antibody was applied overnight at 4°C under light-protected conditions. Afterward, sections were incubated for 2 hr with 0.5% avidin and biotinylated horseradish peroxidase ( Vectastain Elite ABC Kit. Vector Laboratories). Antibody binding was visualized by adding 0.05% diaminobenzidine substrate, 0.3% ammonium nickel sulfate in 0.05 M Trisbase 7-9®, and 0.003% H₂O₂ for <5 min. Then the reaction was stopped by adding PBS (when the brown color was intense enough). Stained sections were placed on object plates and dried. Sections were then exposed to ascending ethanol series, for 10 min in xylene (Sigma-Aldrich) and finally embedded with Entellan®Neu (Merck Millipore, Schwalbach, Germany).

For toluidine blue staining (Sigma-Aldrich), sections were mounted on slides, dried and exposed to descending ethanol series, briefly rinsed in double-distilled water and then incubated in 0.1% toluidine blue working solution (pH 2.3) for 1–3 min. Thereafter, the sections were briefly rinsed in double-distilled water. Ninety-five percent ethanol with traces of glacial acetic acid was used for color differentiation of the staining. Sections were then exposed to 100% ethanol, followed by a 1:1 mixture of 100% ethanol and xylene and finally xylene for 3–10 min. Sections were embedded with Entellan®Neu (Merck Millipore, Schwalbach, Germany). Toluidine blue is a typical Nissl staining that is widely used to assess the cytoarchitecture of brain tissue. Although toluidine blue has been also shown to stain glial cells, neurons can be clearly identified by larger cell body, nucleus, and nucleolus (Zhu, Liu, Zou, & Torbey, 2015).

### 2.5 | Stereological counting of microglia

The numbers of microglia (Iba1-positive cell somata) were estimated with design-based stereology recently described for hippocampal slice cultures in detail (Papageorgiou et al., 2016). In brief, we implemented the optical fractionator probe using the Stereoinvestigator® 5.65 software (MicroBrightField Europe, Delft, The Netherlands), which provides an estimator of the total particle number in a three-dimensional structure. Sequential sections (total of four to seven) of each slice culture were included in the analysis. To satisfy the coefficient of sampling error (CE) < 0.1, the optimal size of the frame-associated area and grid spacing was chosen (Papageorgiou et al., 2016). The estimated microglia number of each slice culture, \( \hat{N} \), was determined using the optical fractionator equation, that is, \( \hat{N} = Q \times \frac{\text{area sampling fraction}}{\text{section height}} \times \frac{\text{sampled area}}{\text{fractionator area}} \). \( Q \) was the number of the counted cells in the fractionator frame-associated area of all sections, \( h sf \) the height sampling fraction, \( \text{ssf} \) the sampling fraction, that is, the interval of sections sampled through an object of interest. As we sampled every section of each slice culture, the section sampling fraction was always 1. As a result of inevitable tissue shrinkage during the staining procedures, the initial section thickness before staining (25 µm, obtained by cutting with a cryostat) had to be adjusted.

Due to very low cell numbers and irregular cell distribution in clodronate-exposed slice cultures (microglial depletion), we defined the fractionator frame-associated area as identical to the contour area of all sections in all clodronate-exposed slice cultures, that is, \( \text{ssf} = 1 \). The estimated microglial number of each slice culture was determined using the optical fractionator equation. The counted cell number was corrected for tissue shrinkage (Papageorgiou et al., 2016). Initially, identification and counting of microglia were made independently by two of the authors, which resulted in similar cell numbers.

### 2.6 | Recording solutions and drugs

Slice cultures were constantly supplied with prewarmed recording solution (artificial cerebrospinal fluid, ACSF). ACSF contained 129 mM NaCl, 3 mM KCl, 1.25 mM NaH₂PO₄, 1.8 mM MgSO₄, 1.6 mM CaCl₂, 21 mM NaHCO₃, and 10 mM glucose (Kann, Huchzermeyer, Kovács, Wirtz, & Schuelke, 2011; Papageorgiou et al., 2016). The pH was 7.3 when the recording solution was saturated with 95% O₂ and 5% CO₂. Recordings were done at 34 ± 1°C.

### TABLE 1 List of antibodies used in the study

| Name                                      | Species          | Manufacturer/Order No/RRID           | Dilution |
|-------------------------------------------|------------------|-------------------------------------|----------|
| Anti-Iba1 (ionized calcium-binding adapter molecule 1) | Rabbit polyclonal | Fujifilm-WAKO/019-19741/AB_839504  | 1:1,000  |
| Anti-Parvalbumin (clone PARV-19)          | Mouse monoclonal | Sigma-Aldrich/P3088/AB_477329       | 1:1,000  |
| Anti-GFAP (glial fibrillary acidic protein) | Rabbit polyclonal | Agilent-Dako/Z0334/AB_447796        | 1:1,000  |
| Anti-MHC-II (MRC OX-6)                    | Mouse monoclonal | Abcam/ab23990/AB_447796             | 1:250    |

**Recording solutions and drugs**

Slice cultures were constantly supplied with prewarmed recording solution (artificial cerebrospinal fluid, ACSF). ACSF contained 129 mM NaCl, 3 mM KCl, 1.25 mM NaH₂PO₄, 1.8 mM MgSO₄, 1.6 mM CaCl₂, 21 mM NaHCO₃, and 10 mM glucose (Kann, Huchzermeyer, Kovács, Wirtz, & Schuelke, 2011; Papageorgiou et al., 2016). The pH was 7.3 when the recording solution was saturated with 95% O₂ and 5% CO₂. Recordings were done at 34 ± 1°C.
Cholinergic gamma oscillations were elicited by continuous application of acetylcholine and the acetylcholine-esterase inhibitor physostigmine via the recording solution (Kann et al., 2011; Ta et al., 2019). Acetylcholine was purchased from Sigma-Aldrich, and physostigmine was purchased from Tocris (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany).

2.7 | Recordings of local field potential and \([K^+]_0\)

For electrophysiological recordings, the intact Biopore™ membrane carrying slice cultures was inserted into the recording chamber (Kann et al., 2011; Papageorgiou et al., 2016). Slice cultures were maintained at the interface between the recording solution and the ambient gas mixture. Intact Biopore™ membrane inserts ensure rapid and efficient supply of oxygen, energy substrates, and drugs through the recording solution (rate 1.8 ml/min) that flows underneath. The interface condition permits constant oxygen supply from the ambient gas mixture (95% \(O_2\) and 5% \(CO_2\), rate 1.5 l/min).

Local field potentials were recorded with glass electrodes (resistance of 1–2 MOhm) that were made from GB150F-8P borosilicate filaments (Science Products GmbH, Hofheim, Germany) using a Zeitz DMZ Puller (Zeitz-Instruments Vertriebs GmbH, Martinsried, Germany), and filled with ACSF. The electrode was positioned in the stratum pyramidale of the CA3 region with a mechanical micro-manipulator (MM 33, Märzhäuser, Wetzlar, Germany). Local field potentials were recorded with an EXT 10-2F amplifier in EPMS-07 housing (npi electronic GmbH, Tamm, Germany), low-pass filtered at 3 kHz, and digitized at 10 kHz using CED 1401 interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK).

Electrical stimulus-induced \([K^+]_0\) transients were recorded with double-barreled \(K^+\)-sensitive and reference (local field potential) glass electrodes manufactured and calibrated as described in detail (Kann et al., 2003; Papageorgiou et al., 2016). The double-barreled electrode was positioned in the stratum pyramidale of the CA1 region. Changes in voltage were digitized at 10 kHz (low-pass filters: 3 kHz for local field potential, 0.3 kHz for \([K^+]_0\)) using CED 1401 interface and Spike2 software. For electrical stimulation with a stimulus isolator (ISO-Flex, A.M.P.I., Jerusalem, Israel), a custom-made, bipolar electrode (25-µm platinum wire) was positioned in the Schaffer collateral pathway to orthodromically activate the CA1 region.

2.8 | Data analysis and statistics

Offline analysis was performed in MatLab 11.0 or R2018B (The MathWorks, Inc., Natick, MA, USA). For stimulus-induced \([K^+]_0\) transients, a modified Nernst equation was used to convert the recorded potentials (mV) to concentration (mM) (Kann et al., 2003; Papageorgiou et al., 2016). For gamma oscillations, data segments of 5 min were subdivided into segments of 30 s, band-pass filtered (FFT filter, pass-band frequency: 5–200 Hz) and processed with Welch’s algorithm and a fast Fourier transformation (FFT size: 8,192). The resulting plots of the power spectral density had a resolution of 1.2207 Hz. For calculation of the time constant, autocorrelations of data segments were fitted with an exponential decay function. Gamma oscillations were analyzed for various parameters, that is, peak frequency (frequency), peak power (power), full width at half maximum, and time constant. Spectrograms (time–frequency plots) were derived from continuous wavelet transforms of a given local field potential recording using Morlet wavelets. This method provides an instant measure of the power of various frequencies at any given time point, thereby offering visualization of fast dynamics in frequency and power.

The sample size is given by “n/N” where “n” refers to the total number of slice cultures (each “slice culture” corresponds to one cultured slice) or membranes carrying slice cultures, and “N” refers to independent preparations, each of which including at least two rat pups. Details about the sample size of each experiment are given in the figure legend. All collected data were included in the results. Data are presented as median ± the interquartile range (IQR = 75% percentile – 25% percentile) with error bars indicating the minimal and the maximal values unless stated otherwise. Statistical significance (*\(p < 0.05\)) was determined in GraphPad Prism 8.0 (GraphPad Software, California, USA). Data distribution was tested for normality with the Shapiro–Wilk test. Statistical tests are specified in the figure legends. Figures were created with MatLab, GraphPad Prism or SigmaPlot (Systat Software, Inc., San Jose, CA, USA), and CorelDRAW (Corel, Ottawa, Ontario, Canada).

3 | RESULTS

Organotypic hippocampal slice cultures of the rat were maintained for 7–12 days in the incubator and exposed to liposome-encapsulated clodronate, the pro-inflammatory T lymphocyte cytokine IFN-γ (type II interferon) or bacterial LPS according to the scheme (Figure 1a). Thereafter, the “conditioned” culture medium was used for biochemical analysis, and the slice cultures were either fixed for histology, including stereological analysis, or transferred for electrophysiological recordings to the interface recording chamber (Kann et al., 2011; Papageorgiou et al., 2016; Schneider et al., 2015). This chamber permits the continuous exchange of recording solution and ambient gas mixture as well as electrophysiological recordings.

3.1 | Microglia in situ

We characterized various features of microglia in situ, that is, in the presence of functional neuronal networks (Figures 1b–d and 2b–d).

Microglia generally lacked the expression of typical activation markers, such as MHC-II, in slice cultures (Figure 1b). As a positive control for microglial reactivity, we stimulated slice cultures with IFN-γ for 72 hr. Indeed, this immunological stimulus evoked...
substantial proliferation of microglia and expression of MHC-II, indicating the transition to a pro-inflammatory phenotype (Figure 1b).

Microglia stained with the marker Iba1 in slice cultures showed small somata (cell bodies) and ramified morphology, including minimal territorial overlap (Figure 1c). Despite locally somewhat increased microglial numbers at the borders, the Iba1 staining pattern was widely similar within each slice. Based on stereological counting of Iba1-positive cells, we estimated a mean population size of about 12,000 microglial cells per slice (Figure 1d).

Exposure of slice cultures to clodronate resulted in effective depletion of microglia (Figure 1c). The residual microglial cells showed a more intense Iba1 staining and appeared to be more hypertrophic. Based on the stereological counting of Iba1-positive cells, we estimated a reduction of the microglial population size by about 96% (Figure 1d).

These findings show that ramified and widely nonreactive microglia are present in slice cultures and that microglia were effectively depleted by liposomal clodronate within days.

3.2 | Hippocampal cytoarchitecture in situ

The cytoarchitecture in hippocampal slice cultures, which were stained with toluidine blue, was well-preserved. In particular, the principal cell layers, such as stratum pyramidale consisting of densely packed somata of glutamatergic pyramidal cells, were well-defined and lacked...
apparent malformations (Figure 2a). Local interneurons stained for the calcium-binding protein parvalbumin were also present and contacted the perisomatic region of pyramidal cells with extensive axon arbors (Figure 2a). Notably, networks of parvalbumin-positive interneurons, such as fast-spiking, GABAergic basket cells, are crucial for the generation of cholinergic gamma oscillations (see below; Gulyás et al., 2010; Kann et al., 2014).

In microglia-depleted slice cultures, there were no obvious effects on the hippocampal cytoarchitecture as well as on the morphology of PV+ interneurons and GFAP+ astrocytes in control and clodronate-exposed slice cultures. The inset (TB, CLOD) shows the part of stratum pyramidale close to the head of the black arrow at higher magnification. (b) The levels of IL-6 were determined in the culture medium after 24 hr. For n/N membranes/preparations (with 3–5 slices per membrane): CTL, 6/3; CLOD, 6/3; LPS (100 ng/ml for 24 hr), 6/3; CLOD + LPS (100 ng/ml for 24 hr), 6/3. *p < 0.0001 (vs. CLOD + LPS). (c) The levels of TNF-α were determined in the culture medium after 24 hr. CTL, 6/3; CLOD, 6/3; LPS (100 ng/ml for 24 hr), 6/3; CLOD + LPS (100 ng/ml for 24 hr), 6/3. *p < 0.0001 (vs. CTL, CLOD, and CLOD + LPS). (d) The levels of nitrite reflecting NO release were determined in the culture medium after 72 hr. CTL, 5/5; CLOD, 4/4; IFN-γ (pooled from 100 ng/ml and 1,000 ng/ml for 72 hr), 12/5. *p = 0.0009 (vs. CTL) and = 0.0011 (vs. CLOD). The data on IFN-γ (positive control) of this experiment were published in Ta et al. (2019). One-way ANOVA with Tukey’s post hoc test (b, c, d). Liposome-containing medium was centrifuged. IFN-γ, interferon-γ; LPS, lipopolysaccharide [Color figure can be viewed at wileyonlinelibrary.com]

3.3 Cytokines and nitric oxide in situ

The levels of the pro-inflammatory cytokines IL-6 and TNF-α were very low and often beneath the detection limit when determined in the culture medium at 24 hr after the medium exchange in slice cultures (Figure 2b,c). The accumulated level of nitric oxide (NO), which was determined by the oxidation product nitrite in the culture medium at 72 hr after the medium exchange, was also low (Figure 2d).

As positive controls for microglial reactivity, we stimulated slice cultures with LPS or IFN-γ for 24 hr and 72 hr, respectively. These immunological stimuli indeed increased the release of IL-6, TNF-α, and NO (Figure 2b–d). Notably, these increases in cytokine and NO
release were moderate compared to paired stimulation with IFN-γ plus LPS (IFN-γ+LPS) that induces severe inflammatory neurodegeneration mainly mediated by reactive and pro-inflammatory microglia (Papageorgiou et al., 2016).

In microglia-depleted slice cultures, the moderate increase in IL-6 and TNF-α release evoked by stimulation with LPS was prevented (Figure 2b,c), further confirming that the ramified microglia can reliably acquire reactive pro-inflammatory phenotypes in situ (Duport & Garthwaite, 2005; Hailer, Jarhult, & Nitsch, 1996; Papageorgiou et al., 2016). Notably, the accumulated low basal level of NO persisted in microglia-depleted slice cultures (Figure 2d). This latter finding might reflect some constitutive NO generation in neurons rather than in glial cells, most likely because of activity of the neuronal NO synthase (nNOS) during synaptic transmission in spontaneously active slice cultures (Hardingham, Dachtler, & Fox, 2013; Huchzermeyer, Berndt, Holzhütter, & Kann, 2013; Ta et al., 2019).

### 3.4 | Cholinergic gamma oscillations in situ

To characterize the functional integrity of local neuronal networks and astrocyte syncytia in slice cultures, we performed electrophysiological recordings.

**FIGURE 3** Recordings of cholinergic gamma oscillations in slice cultures. (a) Left, gamma oscillations were elicited by acetylcholine (2 μM) and physostigmine (400 nM) (ACh + Phy, black bar) at 34 ± 1°C. Middle, local field potential (LFP) recordings were done in stratum pyramidale of the CA3 region; DG, dentate gyrus. Right, gamma oscillations (30–70 Hz) in local neuronal networks emerge from precise mutual synaptic transmission between glutamatergic pyramidal cells (PC) and parvalbumin-positive (PV+) GABAergic interneurons that generate action potentials at 1–3 Hz and >20 Hz ("fast-spiking"), respectively. The axonal connection from PC (excitatory drive) to PV+ is not shown. Note that this is a simplified scheme. (b) Sample spectrograms of gamma oscillations in control (CTL) and clodronate-exposed (CLOD) slice cultures were calculated from individual recordings. Heat-scale colors encode for power in arbitrary units (a.u.). (c) Corresponding frequencies were calculated at 30-s intervals. For n/N slices/preparations: CTL, 24/7; CLOD, 32/8. Frequency: 0.5 min, 2.5 min, 3 min, 4.5 min, and 5 min, p = 0.9443; 1 min, 2 min, and 4 min, p = 0.7990; 1.5 min, p = 0.8991; 3.5 min, p = 0.9165 (CTL vs. CLOD, each). Two-way ANOVA with Holm–Sidak’s post hoc test. Data are presented as mean ± SEM. A, astrocyte; GABA, γ-aminobutyric acid; Glu, glutamate [Color figure can be viewed at wileyonlinelibrary.com]
We first applied the neurotransmitter acetylcholine and recorded local field potential responses (Figure 3a) (Papageorgiou et al., 2016; Schneider et al., 2015). Slice cultures usually show spontaneous asynchronous neuronal network activity in the absence of exogenous neurotransmitter receptor ligands (Huchzermeyer et al., 2013; Neumann et al., 1998). The continuous application of acetylcholine in slices enhances neural excitability and mimics cholinergic input to the hippocampus during exploratory behavior in vivo (Hájos & Paulsen, 2009). Acetylcholine reliably induced persistent gamma oscillations that had a frequency of around 40 Hz and were quite stable over time (Figure 3b,c). Notably, such cholinergic gamma oscillations in situ share many features with gamma oscillations in vivo and require both glutamatergic excitation and fast rhythmic GABAergic inhibition (Hájos & Paulsen, 2009; Kann et al., 2011).

In microglia-depleted slice cultures, gamma oscillations were still present (Figures 3b and 4a). To be able to identify even discrete impairment of gamma oscillations, we performed a detailed analysis of various oscillation properties in each individual recording using time–frequency plots (Figure 4c), power spectra plots (Figure 4b), and autocorrelations. Notably, there were no differences in the stability of gamma oscillations over time (Figure 3c) as well as in the frequency (Figure 4c), the power (Figure 4d), the full width at half maximum (Figure 4e), and the time constant (Figure 4f) of the oscillations. We note that power mainly increases with number and synchronization of postsynaptic currents at gamma frequency whereas full width at half maximum mainly increases with jitter in the timing of postsynaptic currents (Hájos & Paulsen, 2009; Schneider et al., 2019). Switches to pathological network states were also absent during gamma oscillations.

These findings suggest that the ramified and widely nonreactive microglia are not involved in the generation of gamma oscillations in situ.

### 3.5 | Stimulus-induced $[K^+]_o$ transients in situ

We second applied brief electrical stimulation and recorded $[K^+]_o$ responses (Kann et al., 2003; Papageorgiou et al., 2016) (Figure 5a). The stimulus-induced $[K^+]_o$ transients increased with the stimulation intensity and showed a fast overshoot component that was followed by a prolonged undershoot component (Figure 5b,c), similar to those recorded in acute hippocampal slices and the cerebral cortex in vivo (Heinemann & Lux, 1975; Kann et al., 2005; Liotta et al., 2012). The rise of the overshoot is dominated by activity-dependent $K^+$ efflux from neurons; the fall of the overshoot and the undershoot mainly reflect $K^+$ clearance and buffering by neurons and astrocytes, which likely involves $Na^+/K^+$-ATPases, $K^+$ (Kv4.1) channels, and gap junctions (D'Ambrosio, Gordon, & Winn, 2002; Larsen et al., 2014; Seifert, Henneberger, & Steinhäuser, 2018; Sibille, Panasch, & Rouach, 2014; Verkhratsky & Nedergaard, 2018). Thus, stimulus-induced $[K^+]_o$ transients revealed intact neuronal excitability, synaptic transmission, and $K^+$ homeostasis in slice cultures.

---

**FIGURE 4** Properties of cholinergic gamma oscillations in slice cultures. (a, b) Sample traces of gamma oscillations and corresponding power spectral density (PSD) calculated from data segments of 5 min in control (CTL) and clodronate-exposed (CLOD) slice cultures. (c-f) Frequency (f), power, full width at half maximum (FWHM), and time constant (TAU) of gamma oscillations were calculated from power spectral density and autocorrelation in control and clodronate-exposed slice cultures. For n/N slices/preparations: CTL, 24/7; CLOD, 32/8. Frequency, $p = 0.4866$; power, $p = 0.0533$; FWHM, $p = 0.9391$; TAU, $p = 0.9150$ (CTL vs. CLOD, each). t-Test (FWHM) and Mann–Whitney rank sum test (Frequency, Power, TAU)
In microglia-depleted slice cultures, the stimulus-induced \([K^+]_o\) transients were unchanged (Figure 5c). There were no differences in the amplitudes of overshoot and undershoot at various stimulation intensities (Figure 5d,e). Also, switches to pathological network states such as neural burst firing or synchronized epileptic activity that would be promoted by disturbed K⁺ homeostasis were absent during stimulation.

These findings suggest that the ramified and widely nonreactive microglia are not involved in K⁺ clearance and buffering during robust neuronal activation in situ, neither directly nor indirectly by modulating astrocyte functions (Larsen, Stoica, & MacAulay, 2016; Varga et al., 2020).

4 | DISCUSSION

We explored the physiological role of microglia in neuronal homeostasis and neuromodulation in postnatal cortex tissue. Our main finding is that the absence of microglia has no effects on the properties of sensitive gamma oscillations and stimulus-induced \([K^+]_o\) transients.
4.1 | Gamma oscillations and $[\text{K}^+]_o$ transients in situ and in vivo

We tested the functional integrity of the local neuronal network in the hippocampal CA3 region that is intrinsically capable of generating gamma oscillations (30–70 Hz) (Hájos & Paulsen, 2009; Kann et al., 2011). Gamma oscillations require precise chemical and electrical synaptic transmission between excitatory pyramidal cells and inhibitory interneurons (Colgin, 2016; Hájos & Paulsen, 2009). Among the latter, parvalbumin-positive, GABAergic basket cells, which generate action potentials at >20 Hz (“fast-spiking”) and feature unique biophysical and bioenergetic properties, have a key role in the rhythm generation (Gulyás et al., 2010; Kann et al., 2014). Gamma oscillations emerge in many cortical areas of the brain during sensory perception, selective attention, voluntary movement, and memory formation; they support spike-timing and synaptic plasticity (Kann et al., 2014; Mellon et al., 2007; van Vugt, Schulze-Bonhage, Litt, Brandt, & Kahana, 2010).

Gamma oscillations in hippocampal slice cultures require intact cytoarchitecture and interneuron networks (Hasam-Henderson et al., 2018; Kann, Hollnagel, Elzoheiry, & Schneider, 2016; Schneider et al., 2015). They are reliably present after about one week after the preparation from rat pups, roughly corresponding to the second and third postnatal week in vivo (De Simoni, Griesinger, & Edwards, 2003; Huchzermeyer et al., 2013; Tsintsadze, Minlebaev, Suchkov, Cunningham, & Khazipov, 2015). They share key features with gamma oscillations in hippocampal acute slices and the hippocampus in vivo, such as oscillation generation in the CA3 region, average frequency of around 40 Hz and reversal of the local field potential between the pyramidal cell layer (stratum pyramidale) and the apical dendritic layer (stratum radiatum) (Hájos & Paulsen, 2009; Kann et al., 2011; Vodovozov et al., 2018).

Gamma oscillations reflect the overall increase in excitatory and inhibitory membrane currents in neurons (Kann et al., 2014). They associate with high energy expenditure to fuel cellular ion pumps (ATPases) and maintain ion homeostasis and neurotransmitter release (Galow et al., 2014; Schneider et al., 2019). Thus, $[\text{K}^+]_o$ rarely exceeds 0.5 mM from a baseline of 3.0 mM during gamma oscillations reflecting the maintenance of $\text{K}^+$ homeostasis (Kann et al., 2011, 2016; Somjen, 2002), which differs from the larger $[\text{K}^+]_o$ transients induced by artificial repetitive electrical stimulation in situ and in vivo (Heinemann & Lux, 1977; Kann et al., 2003; Liotta et al., 2012). Conversely, gamma oscillations are exquisitely sensitive to metabolic and oxidative stress (Elzoheiry et al., 2019; Huchzermeyer et al., 2008; Papageorgiou et al., 2016).

Gamma oscillations are also modulated by a variety of homeostatic factors, including the intracellular pH (Stenkamp et al., 2001) and the extracellular levels of potassium ions, ATP, metabolites such as lactate and pyruvate, and amino acids such as glycine (Galow et al., 2014; LeBeau et al., 2002; Schulz et al., 2012; Vodovozov et al., 2018). Therefore, gamma oscillations provide a sensitive readout of even discrete neuronal network dysfunction associated with disturbances in neuronal ion and energy homeostasis and/or neuromodulation.

4.2 | Microglia in situ and in vivo

Microglia are very sensitive to danger signals through innate pattern recognition receptors (scavenger and Toll-like receptors, etc.) as well as to homeostatic imbalance in the tissue. For this purpose, they express a large variety of receptors that recognize, for example, neurotransmitters, glutamate, GABA, ATP, etc., neuronal ligands (CD200, fractalkine, etc.), cytokines (TGF-$\beta$, IFN-$\gamma$, etc.), and complement (C5a, C1q complex, etc.) (Deczkowska et al., 2018; Kettenmann et al., 2013; Prinz et al., 2019).

Conversely, microglia are capable to release signaling ligands, such as glutamate, BDNF, TNF-$\alpha$, and ATP, as well as microvesicles (Antonucci et al., 2012; Häusler et al., 2002; Imura et al., 2013; Parkhurst et al., 2013; Piani & Fontana, 1994). In addition, microglia express diverse transporters for ions and energy substrates, including lactate (Moreira et al., 2009; Payne et al., 1997; Zierler et al., 2008). Lactate, for example, might be taken up or released from microglia depending on the microglial metabolic state (Chaussse, Lewen, Poschet, & Kann, 2020; Gundersen et al., 2015; Holland et al., 2018; Nair et al., 2019). However, it is widely unknown whether microglia-to-neuron signaling is present and functionally relevant under physiological (noninflamed) conditions in the postnatal brain (Gundersen et al., 2015; Kettenmann et al., 2013; Prinz et al., 2019; York et al., 2018).

During brain development, microglia are involved in the support of neural and oligodendrocytic precursor cells as well as in the removal of apoptotic neurons and nonfunctional synapses (Aarum et al., 2003; Hagemeyer et al., 2017; Sierra et al., 2010; Squarzoni et al., 2014). Therefore, it has been suggested that microglia might contribute to structural formation and functional remodeling of neuronal networks (Schafer et al., 2012; Sierra et al., 2010; Tremblay, Lowery, & Majewska, 2010; Ziv et al., 2006).

In hippocampal slice cultures, microglia show ramified morphology, homeostatic surveillance markers, and low release of pro-inflammatory cytokines after a few days of recovery from the preparation (Ajmone-Cat et al., 2013; Chausse et al., 2020; Hailer et al., 1996; Papageorgiou et al., 2016). These features reflect a widely nonreactive microglial phenotype. By contrast, stimulation with exogenous and/or endogenous pro-inflammatory ligands, such as LPS and IFN-$\gamma$, reliably induces different microglial phenotypes in situ with varying outcomes on neuronal survival and function, including gamma oscillations (Ajmone-Cat et al., 2013; Papageorgiou et al., 2016; Ta et al., 2019; Vinet et al., 2012).

We used liposomal clodronate to deplete the microglial population within the first week after slice preparation (Papageorgiou et al., 2016; Vinet et al., 2012). Therefore, we primarily depleted microglia at a tissue maturation stage corresponding to the second postnatal week in vivo (De Simoni et al., 2003; Huchzermeyer et al., 2014). Gamma oscillations are also modulated by a variety of homeostatic factors, including the intracellular pH (Stenkamp et al., 2001) and the extracellular levels of potassium ions, ATP, metabolites such as lactate and pyruvate, and amino acids such as glycine (Galow et al., 2014; LeBeau et al., 2002; Schulz et al., 2012; Vodovozov et al., 2018). Therefore, gamma oscillations provide a sensitive readout of even discrete neuronal network dysfunction associated with disturbances in neuronal ion and energy homeostasis and/or neuromodulation.
et al., 2013; Schneider et al., 2015; Tsintsadze et al., 2015). In this situation, the microglial cell population has higher numbers because of developmental aspects (see above) as well as reactions to slice cutting and culture medium (Hailer et al., 1996; Nikodemova et al., 2015; Prinz et al., 2019).

We report that the absence of microglia did not affect the properties of sensitive neuronal gamma oscillations and stimulus-induced \([K^+]_{o}\) transients. This finding is remarkable and suggests that microglia have no major (constitutive) role in neuronal ion and energy homeostasis, including neuromodulation. This supports experimental evidence that microglial depletion did not affect the outcome of selected behavioral tests in mice (Elmore et al., 2014; Rojo et al., 2019). In addition, microglia appear to be less relevant for the moderate tissue repair at the slice cut surfaces as well as for synaptic remodeling and neuronal network formation, at least, during the second and third postnatal week of hippocampal maturation in situ (De Simoni et al., 2003; Kann et al., 2016; Schneider et al., 2015). Our findings also indicate that the absence of microglia in postnatal cortex tissue has no major consequences on the functions of astrocytic syncytia. Astrocytes are important for neuronal signaling by taking up \(K^+\) ions and neurotransmitters, providing neurotransmitter precursors and energy substrates, and releasing gliotransmitters (Kann et al., 2016; Larsen et al., 2016; Perea et al., 2016; Verkhratsky & Nedergaard, 2018). Microglial depletion might thus be a useful tool for pharmacological interventions in neurological disorders (Bennett & Bennett, 2019; Han, Zhu, Zhang, & Harris, 2019).

We applied local field potential recordings that integrate mainly synaptic transmembrane currents and have a spatial reach of a few hundred micrometers around the electrode tip (Einevoll, Kayser, Logothetis, & Panzeri, 2013). Therefore, our data on gamma oscillations on the network level cannot exclude that the absence of microglia might have contributed to subtle alterations in intrinsic membrane properties, synaptic connectivity, and/or neurotransmission of glutamatergic and GABAergic neurons (Antonucci et al., 2012; Ji, Akgul, Wollmuth, & Tsirka, 2013; Parkhurst et al., 2013; Pribiag & Stellwagen, 2013). However, the presence of regular gamma oscillations and stimulus-induced \([K^+]_{o}\) transients argues for widely intact neuronal and astrocytic functions that are crucial for cortical information processing (Colgin, 2016; Hájos & Paulsen, 2009; Kann et al., 2014; Rasmussen, O’Donnell, Ding, & Nedergaard, 2020).

Our data support the biological concept that nonreactive (surveying) microglia are dispensable for neuronal homeostasis, signaling, and rhythm generation in the healthy postnatal brain. This evolutionary feature might permit microglia to use the enormous phenotypic flexibility, which often includes local proliferation and migration, in response to any homeostatic imbalance and pathology within the CNS.

DECLARATION OF TRANSPARENCY

The authors, reviewers and editors affirm that in accordance to the policies set by the Journal of Neuroscience Research, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

ACKNOWLEDGMENTS

The authors thank Michal Schwartz, Amit Agarwal, Thomas Blank, and Marco Prinz for helpful discussions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors take responsibility for the integrity of the data and the accuracy of the data analysis. Conceptualization, A.L., T.C., I.E.P., B.C., and O.K.; Investigation, A.L., T.-T.T., I.E.P., and B.C.; Formal Analysis, A.L., T.-T.T., I.E.P., J.-O.H., and B.C.; Writing – Original Draft, O.K.; Writing – Review & Editing, A.L., T.-T.T., T.C., I.E.P., J.-O.H., B.C., and O.K.; Visualization, A.L., J.-O.H., and O.K.; Supervision, O.K.; Project Administration, A.L. and O.K.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/jnr.24689.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Ismini E. Papageorgiou https://orcid.org/0000-0001-5810-0483
Oliver Kann https://orcid.org/0000-0003-4365-8067

REFERENCES

Aarum, J., Sandberg, K., Haeberlein, S. L., & Persson, M. A. (2003). Migration and differentiation of neural precursor cells can be directed by microglia. Proceedings of the National Academy of Sciences of the United States of America, 100(26), 15983–15988. https://doi.org/10.1073/pnas.2237050100

Ajmone-Cat, M. A., Mancini, M., De Simone, R., Cilli, P., & Minghetti, L. (2013). Microglial polarization and plasticity: Evidence from organotypic hippocampal slice cultures. Glia, 61(10), 1698–1711. https://doi.org/10.1002/glia.22550

Antonucci, F., Turola, E., Riganti, L., Caleo, M., Gabrielli, M., Perrotta, C., ... Verderio, C. (2012). Microvesicles released from microglia stimulate synaptic activity via enhanced sphingolipid metabolism. The EMBO Journal, 31(5), 1231–1240. https://doi.org/10.1038/emboj.2011.489

Bennett, M. L., & Bennett, F. C. (2019). The influence of environment and origin on brain resident macrophages and implications for therapy. Nature Neuroscience, 23(2), 157–166. https://doi.org/10.1038/s41596-019-0545-6

Bordt, E. A., Ceaserine, A. M., & Bilbo, S. D. (2020). Microglia and sexual differentiation of the developing brain: A focus on ontogeny and intrinsic factors. Glia, 68(6), 1085–1099. https://doi.org/10.1002/glia.23753

Chausse, B., Lewen, A., Poschet, G., & Kann, O. (2020). Selective inhibition of mitochondrial respiratory complexes controls the transition
of microglia into a neurotoxic phenotype in situ. Brain, Behavior, and Immunity. https://doi.org/10.1016/j.bbi.2020.05.052
Colgin, L. L. (2016). Rhythms of the hippocampal network. Nature Reviews Neuroscience, 17(4), 239–249. https://doi.org/10.1038/nrn.2016.21
Colonna, M., & Butovsky, O. (2017). Microglia function in the central nervous system during health and neurodegeneration. Annual Review of Immunology, 35, 441–468. https://doi.org/10.1146/annurev-immunol-051116-052358
Coull, J. A. M., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., ... Coull, J. A. M., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., ... Deczkowska, A., Amit, I., & Schwartz, M. (2018). Microglia: Active sensor and versatile effector cells in the normal and pathologic brain. Nature Neuroscience, 10(11), 1387–1394. https://doi.org/10.1038/nn1997
Hardingham, N., Dachtler, J., & Fox, K. (2013). The role of nitric oxide in pre-synaptic plasticity and homeostasis. Frontiers of Cellular Neuroscience, 7, 190. https://doi.org/10.3389/fncel.2013.00119
Hasam-Henderson, L. A., Gotti, G. C., Mishto, M., Klisch, C., Gerevich, Z., Geiger, J. R. P., & Kovács, R. (2018). NMDA-receptor inhibition and oxidative stress during hippocampal maturation differentially alter parvalbumin expression and gamma-band activity. Scientific Reports, 8(1), 9545. https://doi.org/10.1038/s41598-018-27830-2
Häusler, K. G., Prinz, M., Nolte, C., Weber, J. R., Schumann, R. R., Kettenmann, H., & Hanisch, U.-K. (2002). Interferon-γ differentially modulates the release of cytokines and chemokines in lipopolysaccharide- and pneumococcal cell wall-stimulated mouse microglia and macrophages. European Journal of Neuroscience, 16(11), 2113–2122. https://doi.org/10.1046/j.1460-9568.2002.02287.x
Heinemann, U., & Lux, H. D. (1975). Undershoots following stimulus-induced rises of extracellular potassium concentration in cerebral cortex of cat. Brain Research, 93(1), 63–76. https://doi.org/10.1016/0006-8993(75)90286-3
Heinemann, U., & Lux, H. D. (1977). Ceiling of stimulus induced rises in extracellular potassium concentration in the cerebral cortex of cat. Brain Research, 120(2), 231–249. https://doi.org/10.1016/0006-8993(77)90903-9
Heppner, F. L., Roth, K., Nitsch, R., & Hailer, N. P. (1998). Vitamin E induces ramification and downregulation of adhesion molecules in cultured microglial cells. Glia, 22(2), 180–188. https://doi.org/10.1002/(SICI)1098-1136(199802)22:2<180::AID-Glia3.0.CO;2-B
Holland, R., McIntosh, A. L., Finucane, O. M., Mela, V., Rubio-Araiz, A., Timmons, G., ... Lynch, M. A. (2018). Inflammatory microglia are glycolytic and iron retentive and typify the microglia in APP/PS1 mice. Brain, Behavior, and Immunity, 68, 183–196. https://doi.org/10.1016/j.bbi.2017.10.017
Huchzermeier, C., Albus, K., Gabriel, H.-J., Otáhal, J., Taubenberger, N., Heinemann, U., ... Kann, O. (2013). Oxygen consumption rates during three different neuronal activity states in the hippocampal CA3 network. Journal of Cerebral Blood Flow & Metabolism, 33(2), 263–271. https://doi.org/10.1038/jcbfm.2012.165
Imura, Y., Morizawa, Y., Komatsu, R., Shibata, K., Shinozaki, Y., Kasai, H., ... Koizumi, S. (2013). Microglia release ATP by exocytosis. Glia, 61(8), 1320–1330. https://doi.org/10.1002/glia.22517
Ji, K., Akgül, G., Wollmuth, L. P., & Tsirka, S. E. (2013). Microglia actively regulate the number of functional synapses. PLoS One, 8(2), e56293. https://doi.org/10.1371/journal.pone.0056293
Kann, O., Hollnagel, J.-O., Elzoheiry, S., & Schneider, J. (2016). Energy and potassium ion homeostasis during gamma oscillations. Glia, 64(11), 1713–1726. https://doi.org/10.1002/glia.23529
Frontiers in Molecular Neuroscience, 9, 47. https://doi.org/10.3389/fnmol.2016.00047

Kann, O., Huchzermeyer, C., Kovács, R., Wirtz, S., & Schuelke, M. (2011). Gamma oscillations in the hippocampus require high complex I gene expression and strong functional performance of mitochondria. Brain, 134(Pt 2), 345–358. https://doi.org/10.1093/brain/awq333

Kann, O., Kovács, R., Njunting, M., Behrens, C. J., Otáhal, J., Lehmann, T. N., & Heinemann, U. (2005). Metabolic dysfunction during neuronal activation in the ex vivo hippocampus from chronic epileptic rats and humans. Brain, 128(Pt 10), 2396–2407. https://doi.org/10.1093/brain/awh568

Kann, O., Papageorgiou, I. E., & Draguhn, A. (2014). Highly energized inhibitory interneurons are a central element for information processing in cortical networks. Journal of Cerebral Blood Flow & Metabolism, 34(8), 1270–1282. https://doi.org/10.1038/jcbfm.2014.104

Kann, O., Schuchmann, S., Buchheim, K., & Heinemann, U. (2003). Coupling of neuronal activity and mitochondrial metabolism as revealed by NAD(P)H fluorescence signals in organotypic hippocampal slice cultures of the rat. Neuroscience, 119(1), 87–100. https://doi.org/10.1016/S0306-4522(03)00026-5

Kettenmann, H., Kirchhoff, F., & Verkhatsky, A. (2013). Microglia: New roles for the synaptic stripper. Neuron, 77(1), 10–18. https://doi.org/10.1016/j.neuron.2012.12.023

Larsen, B. R., Assentoft, M., Cotrina, M. L., Hua, S. Z., Nedergaard, M., Kaila, K., … MacAulay, N. (2016). Managing brain extracellular K+ during neuronal activity: The physiological role of the Na+/K+ -ATPase subunit isoforms. Frontiers in Physiology, 7, 141. https://doi.org/10.3389/fphys.2016.00141

LeBeau, F. E. N., Towers, S. K., Traub, R. D., Whittington, M. A., & Buhl, E. H. (2002). Fast network oscillations induced by potassium transients in the rat hippocampus in vitro. The Journal of Physiology, 542(1), 167–179. https://doi.org/10.1113/jphysiol.2002.015933

Liotta, A., Röser, J., Huchzermeyer, C., Wojtowicz, A., Kann, O., Schmitz, D., … Kovács, R. (2012). Energy demand of synaptic transmission at the hippocampal Schaffer-collateral synapse. Journal of Cerebral Blood Flow & Metabolism, 32(11), 2076–2083. https://doi.org/10.1038/jcbfm.2012.116

Melloni, L., Molina, C., Pena, M., Torres, D., Singer, W., & Rodríguez, E. (2007). Synchronization of neural activity across cortical areas correlates with conscious perception. The Journal of Neuroscience, 27(11), 2858–2865. https://doi.org/10.1523/JNEUROSCI.4623-06.2007

Moreira, T. J., Pierre, K., Maekawa, F., Repond, C., Cebere, A., Liljequist, N., Nair, S., Sobotka, K. S., Joshi, P., Gressens, P., Fleiss, B., Thornton, C., … Steinhäuser, C. (2017). Activity-dependent switch of GABAergic inhibition into glutamatergic excitation in astrocyte-neuron networks. Elife, 5, e20362. https://doi.org/10.7554/eLife.20362

Pini, D., & Fontana, A. (1994). Involvement of the cytosine transport system xc- in the macrophage-induced glutamate-dependent cytotoxicity to neurons. The Journal of Immunology, 152(7), 3578–3585.

Pribiag, H., & Stellwagen, D. (2013). TNF-α downregulates inhibitory neurotransmission through protein phosphatase 1-dependent trafficking of GABA_A receptors. The Journal of Neuroscience, 33(40), 15879–15893. https://doi.org/10.1523/JNEUROSCI.0530-13.2013

Prinz, M., Jung, S., & Priller, J. (2019). Microglia biology: One century of evolving concepts. Cell, 179(2), 292–311. https://doi.org/10.1016/j.cell.2019.08.053

Ransohoff, R. M., & Perry, V. H. (2009). Microglial physiology: Unique stimuli, specialized responses. Annual Review of Immunology, 27, 119–145. https://doi.org/10.1146/annurev.immunol.021908.132528

Rasmussen, R., O’Donnell, J., Ding, F., & Nedergaard, M. (2020). Interstitial ions: A key regulator of state-dependent neural activity? Progress in Neurobiology, 193, 101802. https://doi.org/10.1016/j.pneurobio.2020.101802

Rojo, R., Raper, A., Ozdemir, D. M., Lefevre, L., Grabert, K., Wollscheid-Lengeling, E., … Prudins, C. (2019). Deletion of a Csf1r enhancer selectively impacts Csf1r expression and development of tissue macrophage populations. Nature Communications, 10(1), 3215. https://doi.org/10.1038/s41467-019-11053-8

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., … Stevens, B. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron, 74(4), 691–705. https://doi.org/10.1016/j.neuron.2012.03.026

Schneider, J., Berndt, N., Papageorgiou, I. E., Maurer, J., Bulik, S., Both, M., … Kann, O. (2019). Local oxygen homeostasis during various neuronal network activity states in the mouse hippocampus. Journal of Cerebral Blood Flow & Metabolism, 39(5), 859–873. https://doi.org/10.1177/0271678X17740091

Schneider, J., Lewen, A., Ta, T.-T., Galow, L. V., Isola, R., Papageorgiou, I. E., & Kann, O. (2015). A reliable model for gamma oscillations in hippocampal tissue. Journal of Neuroscience Research, 93(7), 1067–1078. https://doi.org/10.1002/jnr.23590

Schulz, S. B., Klaft, Z.-J., Rössler, A. R., Heinemann, U., & Gerevich, Z. (2012). Purinergic P2X, P2Y and adenosine receptors differentially modulate hippocampal gamma oscillations. Neuropharmacology, 62(2), 914–924. https://doi.org/10.1016/j.neuropharm.2011.09.024

Seifert, G., Henneberger, C., & Steinhäuser, C. (2018). Diversity of astrocyte potassium channels: An update. Brain Research Bulletin, 136, 26–36. https://doi.org/10.1016/j.brainresbull.2016.12.002
Sibille, J., Pannasch, U., & Rouach, N. (2014). Astroglial potassium clearance contributes to short-term plasticity of synaptically evoked currents at the tripartite synapse. The Journal of Physiology, 592(1), 87–102. https://doi.org/10.1113/jphysiol.2013.261735
Sierra, A., Encinas, J. M., Deudero, J. J. P., Chancey, J. H., Enikolopov, G., Overstreet-Wadiche, L. S.,... Maletic-Savatic, M. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. Cell Stem Cell, 7(4), 483–495. https://doi.org/10.1016/j.stem.2010.08.014
somjen, G. G. (2002). Ion regulation in the brain: Implications for pathophysiology. The Neuroscientist, 8(3), 254–267. https://doi.org/10.1177/1073858402008003011
Squarzoni, P., Oller, G., Hoefeli, G., Pont-Lezica, L., Rostaing, P., Low, D.,... Garel, S. (2014). Microglia modulate wiring of the embryonic forebrain. Cell Reports, 8(5), 1271–1279. https://doi.org/10.1016/j.celrep.2014.07.042
Stellwagen, D., & Malenka, R. C. (2006). Synaptic scaling mediated by glial TNF-α. Nature, 440(7087), 1054–1059. https://doi.org/10.1038/nature04671
Stenkamp, K., Palva, J. M., Uusisaari, M., Schuchmann, S., Schmitz, D., Heinemann, U., & Kaila, K. (2001). Enhanced temporal stability of cholinergic hippocampal gamma oscillations following respiratory alkalosis in vitro. Journal of Neurophysiology, 85(5), 2063–2069. https://doi.org/10.1152/jn.2001.85.5.2063
Stoppani, L., Buchs, P.-A., & Muller, D. (1991). A simple method for organotypic cultures of nervous tissue. Journal of Neuroscience Methods, 37(2), 173–182. https://doi.org/10.1016/0165-0270(91)90128-m
Ta, T.-T., Dikmen, H. O., Schilling, S., Chause, B., Lewen, A., Hollnagel, J.-O., & Kann, O. (2019). Priming of microglia with IFN-γ slows neuronal gamma oscillations in situ. Proceedings of the National Academy of Sciences of the United States of America, 116(10), 4637–4642. https://doi.org/10.1073/pnas.1813562116
Tremblay, M. E., Lowery, R. L., & Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. PLoS Biology, 8(11), e1000527. https://doi.org/10.1371/journal.pbio.1000527
Tsintsadze, V., Minlebaev, M., Suchkov, D., Cunningham, M. O., & Khazipov, R. (2015). Ontogeny of kainate-induced gamma oscillations in the rat CA3 hippocampus in vitro. Frontiers in Cellular Neuroscience, 9, 195. https://doi.org/10.3389/fncel.2015.00195
van Vugt, M. K., Schulze-Bonhage, A., Litt, B., Brandt, A., & Kahana, M. J. (2010). Hippocampal gamma oscillations increase with memory load. The Journal of Neuroscience, 30(7), 2694–2699. https://doi.org/10.1523/JNEUROSCI.0567-09.2010
Varga, D. P., Menyhárt, Á., Pösfai, B., Császár, E., Lénárt, N., Cserép, C.,... Dénes, Á. (2020). Microglia alter the threshold of spreading depolarization and related potassium uptake in the mouse brain. Journal of Cerebral Blood Flow & Metabolism. https://doi.org/10.1177/0271263X20784920
Verkhratsky, A., & Nedergaard, M. (2018). Physiology of astroglia. Physiological Reviews, 98(1), 239–389. https://doi.org/10.1152/physrev.00042.2016
Vinet, J., van Weering, H. R. J., Heinrich, A., Källin, R. E., Wegner, A., Brouwer, N.,... Biber, K. (2012). Neuroprotective function for ramified microglia in hippocampal excitotoxicity. Journal of Neuroinflammation, 9, 27. https://doi.org/10.1186/1742-2094-9-27
Vodovozov, W., Schneider, J., Elzoheiry, S., Hollnagel, J.-O., Lewen, A.,... Kann, O. (2018). Metabolic modulation of neuronal gamma-band oscillations. Pflügers Archiv—European Journal of Physiology, 470(9), 1377–1389. https://doi.org/10.1007/s00424-018-2156-6
York, E. M., Bernier, L.-P., & MacVicar, B. A. (2018). Microglial modulation of neuronal activity in the healthy brain. Developmental Neurobiology, 78(6), 593–603. https://doi.org/10.1002/dneu.22571
Yousif, N. M., de Oliveira, A. C. P., Brioschi, S., Huell, M., Biber, K., & Fiebich, B. L. (2018). Activation of EP3 receptor suppresses poly(I:C) and LPS-mediated inflammation in primary microglia and organotypic hippocampal slice cultures: Contributing role for MAPKs. Glia, 66(4), 708–724. https://doi.org/10.1002/glia.23276
Zhu, Y., Liu, F., Zou, X., & Torbey, M. (2015). Comparison of unbiased estimation of neuronal number in the rat hippocampus with different staining methods. Journal of Neuroscience Methods, 254, 73–79. https://doi.org/10.1016/j.jneumeth.2015.07.022
Zierler, S., Frei, E., Grissmer, S., & Kerschbaum, H. H. (2008). Chloride influx provokes lamellipodium formation in microglial cells. Cellular Physiology and Biochemistry, 21(1–3), 55–62. https://doi.org/10.1159/000113747
Ziv, Y., Ron, N., Butovsky, O., Landa, G., Sudai, E., Greenberg, N.,... Schwartz, M. (2006). Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. Nature Neuroscience, 9(2), 268–275. https://doi.org/10.1038/nn1629

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.
Transparent Peer Review Report
Transparent Science Questionnaire for Authors

How to cite this article: Lewen A, Ta T-T, Cesetti T, et al. Neuronal gamma oscillations and activity-dependent potassium transients remain regular after depletion of microglia in postnatal cortex tissue. J Neurosci Res. 2020;98:1953–1967. https://doi.org/10.1002/jnr.24689