Synaptic development is regulated by microglial THIK-1 K⁺ channels

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Edited by Lily Yeh Jan, HHMI and University of California, San Francisco, CA, and approved September 8, 2021 (received for review April 6, 2021)

Microglia are the resident immune cells of the central nervous system. They constantly survey the brain parenchyma for redundant synapses, debris, or dying cells, which they remove through phagocytosis. Microglial ramification, motility, and cytokine release are regulated by tonically active THIK-1 K⁺ channels on the microglial plasma membrane. Here, we examined whether these channels also play a role in phagocytosis. Using pharmacological blockers and THIK-1 knockout (KO) mice, we found that a lack of THIK-1 activity approximately halved both microglial phagocytosis and marker levels for the lysosomes that degrade phagocytically removed material. These changes may reflect a decrease of intracellular Ca²⁺ activity, which was observed when THIK-1 activity was reduced, since buffering Ca²⁺ reduced phagocytosis. Less phagocytosis is expected to result in impaired pruning of synapses. In the hippocampus, mice lacking THIK-1 expression had an increased number of anatomically and electrophysiologically defined glutamatergic synapses during development. This resulted from an increased number of presynaptic terminals, caused by impaired removal by THIK-1 KO microglia. The dependence of synaptic number on THIK-1 K⁺ channels, which control microglial surveillance and phagocytic ability, implies that changes in the THIK-1 expression level in disease states may contribute to altering neural circuit function.

Significance

Microglia are the brain’s resident immune cells, surveying the brain with motile processes, which can remove pathogens but also prune unnecessary junctions between the neurons (synapses). A potassium channel, THIK-1, in the microglial membrane allows efflux of potassium from these cells and thereby regulates their membrane voltage as well as their process motility and release of inflammatory mediators. Here, using THIK-1–blocking drugs and THIK-1–deficient mice, we demonstrate that THIK-1 controls removal of synaptic material by microglia, which reduces the number of functional synapses in the developing brain.

Author contributions: P.I. and D.A. designed research; P.I., H.S., and C.H. performed research; P.I. contributed new reagents/analytic tools; P.I., H.S., and G.G. analyzed data; and P.I., H.S., and D.A. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2106294118/DCSupplemental.

Published October 12, 2021.

PNAS 2021 Vol. 118 No. 42 e2106294118
https://doi.org/10.1073/pnas.2106294118 | 1 of 10
controlling microglial phagocytosis may have therapeutic value in the mature brain.

Materials and Methods

Rodent Procedures. All animal procedures were performed in accordance with the UK Animals (Scientific Procedures) Act 1986 (Home Office License 70/8976), with the approval of the University College London Animal Welfare and Ethical Review Body. Rodents were maintained on a 12-h light/dark cycle, and food and water were available ad libitum. Mice (postnatal day P17 to P19, P26 to P32, and P120 to 130) were housed in individually ventilated cages, and Sprague–Dawley rats (P12 to 13) were kept in open-shelf units. Animals of both sexes were euthanized by cervical dislocation followed by decapitation or by an overdose of pentobarbital sodium (Euthatal, 200 mg/kg body weight) injected intraperitoneally before transcardial perfusion of tissues with 4% paraformaldehyde (PFA).

For mouse experiments, THIK-1 knockouts (Kcnk13-I1m1-Em1-B6) were generated by Medical Research Council Harwell as previously described in detail (3). Briefly, a single nucleotide insertion in the gene encoding the THIK-1 channel protein (Kcnk13) leads to a premature stop codon, producing a truncated protein that fails to form a channel.

For Ca2+ imaging, GCaMP5g-ires-tdTomato transgenic mice (27) were crossed with Cx3cr1CreER mice (28), which allowed expression of GCaMP5g and tdTomato in microglia at least 21 d following tamoxifen gavage (Sigma T5648, 120 μg/g body weight for 4 consecutive days). Only Cx3cr1 heterozygotes were used for experiments.

Acute Brain Slicing. Dorsal hippocampal slices (parasagittal, 250 μm) were prepared on a Leica VT1200S vibratome in ice-chilled slicing solution containing 124 NaCl, 2.5 KCl, 26 NaHCO3, 1 NaH2PO4, 10 glucose (mOsM and pH set to 7.4 when bubbled with 5% CO2). T5648, 120 μg/g body weight for 4 consecutive days). Only Cx3cr1 heterozygotes were used for experiments.

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Microglial Ca2+ detection with primary antibodies in blocking buffer for 12 h at 4°C. Following three 10-min washes in 0.1% Tween-20 in PBS, membranes were incubated for 1 h at room temperature in horseradish peroxidase-conjugated secondary antibodies diluted 1:10,000 in blocking solution. Following three 10-min washes, signals were detected with chemiluminescent substrate (Thermo Fisher 34075) with an ImageQuant LAS 4000 camera. Bands were quantified using the Gels tool in ImageJ/FIJI, and values were normalized for loading using p-actin (1:5,000, Proteintech 66009).
Apochromat 63×1.4 objective. Three confocal images from the CA1 stratum radiatum region (at 1.5-μm intervals) were taken per brain slice, and five brain slices were taken per animal. Images were analyzed individually and then averaged across stacks and brain slices to obtain animal means. After background substraction (with a 10-pixel rolling ball average), marker areas were quantified using a custom-based intensity threshold protocol with ImageJ/FIJI, and the Analyze Particles function was used to quantify puncta number and area. For thresholding, sample images were first manually thresholded for each channel blinded to condition and genotype, and a suitable range above threshold was established, which was then kept constant throughout (Bassoon: 15–25; Homer1: 30–255; images were 8-bit). Size exclusion (<1.2 μm2 and <0.05 μm2) was applied to exclude any objects unlikely to represent synapti- c apt puncta. Synapses were defined by the presence of overlapping presynaptic-post synaptic puncta (33). Presynaptic colocalization with microglia was analyzed as the total area of Bassoon puncta within the Iba1-stained cell area after thresholding. All imaging and analysis were done with the researcher blind to genotype or treatment.

**Cell Density Analysis.** Brain slices were imaged (640 μm × 640 μm) using a Zeiss LSM700 microscope with a Plan-Apochromat 20x/0.8 objective. Cell density and spatial distribution were analyzed as in ref. 34. Briefly, cells were counted to perform cell density as well as the average nearest-neighbor distance (sed) and their regularity index. The latter is the ratio of the mean NND to the SD of the NND for the whole population of cells and describes how regular the spacing of microglia is. One maximum-projected z-stack (3-μm depth) from the CA1 stratum radiatum region was analyzed per slice, and five slices were averaged per animal.

**Electrophysiology.** Slices were individually transferred to the recording chamber and perfused at 3 to 5 mL/min with aCSF, which was maintained at 32 to 34°C. Pyramidal neurons in the CA1 region of the dorsal hippocampus were selected visually using an Olympus 60×1.4 water-immersion objective in combination with differential interference contrast optics. Cells were recorded in the whole-cell, voltage-clamp configuration with glass patch-pipettes (resistance in the bath solution 2 to 4 MΩ). Junction potentials (−10 to −40 mV) were corrected for. Recorded signals were sampled and digitized at 20 kHz and then further filtered offline at 2 kHz for analysis and data presentation. During the entire course of recording, access resist- ance was monitored by periodically applying a −5-mV voltage pulse. Cells were excluded from analysis if the access resistance changed by more than 20% during the course of an experiment.

To study excitatory synapses, pipettes were filled with internal solution containing the following (in millimolar): 123.2 K-glucuronate, 7.7 KCl, 4 NaCl, 0.5 CaCl2, 10 HEPES, 5 EGTA, 4 MgATP, and 0.5 NaGTP (pH 7.2 to 7.3). The calculated reversal potential for Cl− (ECl) with these solutions was −62 mV, and a holding potential (Vh) of −65 mV was used. Thus, at Vh = −65 mV, CI−-mediated inhibitory postsynaptic currents (IPSCs) were nearly invisible, and cation-mediated excitatory postsynaptic currents (EPSCs) were inward. To isolate single vesicular events, 500 nM TTX was applied, and the frequency and amplitude of EPSCs in TTX were monitored. To assess whole-cell glutamate receptor-mediated currents, cells were whole-cell clamped and voltage-clamped at −40 mV, and recorded in the presence of the GABAAR antagonist Gabazine (100 μM, Tocris 1262). N-methyl-D-aspartic acid (NMDA, 10 μM, Tocris 0114) or kainic acid (Tocris, 1 μM, Tocris 0222) were bath applied sequentially (ensuring that holding current returned to the control current level before subsequent drug application, 10 to 15 min), and the resulting change in current was measured. Peak shifts in holding current induced by each drug were reported as the change in mean current in 20-s segments for control solution and at the peak of the NMDA and kainic acid applications by making histograms of all data points and then fitting a Gaussian distribu- tion to each histogram to define the mean current (using Clampfit 10.4; Molecular Devices).

**Statistics.** Data are presented as mean ± SEM. Data normality was assessed using the D’Agostino–Pearson test. Statistical significance (taken as P < 0.05) was assessed using unpaired two-tailed Student’s t tests (Figs. 1 E, H, and J, 2G, 3 B–G and I, and 4 C, F, and G and SI Appendix, Figs. S4D and S8 B, C, and E), Mann–Whitney U tests (Figs. 4 B, D, and E and SI Appendix, Figs. S3, S4B, S55–S57, and S8D), or one-way ANOVA followed by Dunnett’s post hoc tests for individual comparisons (Figs. 1C and 2 and E and SI Appendix, Fig. S2). All statistical analysis was performed in Microsoft Excel 2016, GraphPad Prism 7, and SigmaPlot 11.

**Results**

**Microglial Phagocytosis Is Regulated by K+ Channels.** Ion channels and receptors controlling microglial motility might be involved in phagocytosis. These include P2Y12 receptors that regulate ATP-evoked microglial chemotaxis to an injury site (35) and THIK-1 K+ channels that regulate microglial morphology and surveillance (3). It was previously reported that P2Y12 receptors regulate phagocytosis (36, 37), but the role of microglial K+ channels is unknown. Therefore, we first tested whether blocking THIK-1 (the dominant K+ channel expressed in “resting” microglia) affects microglial phagocytosis. We studied phagocytosis in situ in brain slices, because microglia in slices retain their normal morphology and interactions with surrounding cells, which they lose in vitro, and critically, cultured microglia rapidly change their protein expression profiles (38) and do not express THIK-1 (Kcnk13) K+ channels (39), which are the main channels setting the resting potential in microglia in vivo (3). Following a recovery period of a few hours after brain slicing, which allows some microglial activation to occur (29), fluorophore-labeled resin microbeads were applied (30, 31) onto rat hippocampal slices in the presence or absence of pharmacological blockers (Fig. L4).

Microglia engulf and phagocyte their substrates thanks to membrane protrusions and phagocytic cups, the formation of which relies upon Ca2+-dependent cytoskeletal rearrangements (40–42). Indeed, microglia were seen to form phagocytic cups and engulf microbeads in situ (SI Appendix, Fig. S1). As negative control experiments, the brain slices were incubated at 4°C or in the presence of the actin inhibitor cytochalasin D, both of which disrupt cytoskeletal dynamics (43). We found that both conditions potently blocked phagocytosis (SI Appendix, Fig. S2). MRS2578, a blocker of the P2Y2 receptor known to regulate microglial phagocytosis (44, 45), also reduced the fraction of microglia that was phagocytic (SI Appendix, Fig. S2).

Potassium efflux across the microglial membrane via THIK-1 was previously found to control NLRP3 activation (3, 46). We found that elevating the extracellular K+ concentration ([K+]o) to prevent such efflux and depolarize the cells reduced the fraction of microglia that were phagocytic by a third (Fig. 1 B and C). This was done in the presence of TTX to block voltage-gated Na+ channels and thus action potential-evoked synaptic transmitter release from neurons (TTX had no effect by itself: Fig. 1C). To determine how the raised [K+]o altered phagocytosis, we applied blockers of different K+ channel types. Blocking Ca2+-activated K+ channels with charybdoxotoxin had no significant effect, but both the K+ channel blockers bupivacaine and tetrodopylaminommonium (TPA) inhibited phagocytosis (Fig. 1 B and C). Since bupivacaine blocks both two-pore domain channels and voltage-gated Na+ channels while TPA blocks both two-pore domain channels and voltage-gated K+ channels (47, 48), these data are consistent with K+ efflux via THIK-1 (or down-stream changes in microglial membrane voltage, Vm) regulating phagocytosis. In addition to pharmacology, we also used a mouse in which THIK-1 was knocked out (KO) and found that deletion

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https://doi.org/10.1073/pnas.2106294118
of THIK-1 had a similar inhibitory effect on microglial phagocytosis (Fig. 1 D–F). Differences may arise between chemically blocking a channel and deleting it genetically (which may have a stronger effect when the chemical block is not complete but may also suffer from compensatory changes). In addition, species differences (i.e., between rat and mouse) may play a role. However, no significant difference was found between the decrease in phagocytic ability induced by chemical and genetic removal of THIK-1 currents (one-way ANOVA, \( P = 0.2 \)).

Lastly, we assessed whether protein levels of lysosome markers were affected in the THIK-1 KO mice (since phagocytosed particles are ultimately passed to lysosomes for digestion). Levels of the microglial lysosome marker CD68 were approximately halved in hippocampal homogenates from KO mice (Fig. 1 G and H), while the homologous lysosome marker LAMP1, which is also expressed by other brain cells including astrocytes and oligodendrocytes (49), remained unchanged (Fig. 1 I and J), indicating a specific effect of THIK-1 KO on the lysosomes that degrade material phagocytosed by microglia.

**THIK-1 Controls Ca\(^{2+}\) Activity in Microglia.** Intracellular calcium concentration ([Ca\(^{2+}\)]) has been suggested to regulate phagocytosis (41, 42, 44, 50). To further understand how THIK-1 controls microglial phagocytic ability, we imaged Ca\(^{2+}\) in microglia from Cx3cr1\(^{CreER}\) × GCaMP5g-IRES-tdTomato mice, which express the genetically encoded Ca\(^{2+}\) indicator GCaMP5g in a tamoxifen-inducible manner (Fig. 2A). Phagocytic cells exhibit periodic spontaneous Ca\(^{2+}\) transients as well as stimulus-evoked Ca\(^{2+}\) rises (51). Consistently with previous work (52), microglia showed a low frequency of spontaneous Ca\(^{2+}\) activity.
Fig. 2. Two-pore domain K⁺ channels regulate Ca²⁺ activity in microglia. (A) Tamoxifen-induced microglia from Cx3cr1CreER × GCaMP5g-IRES-tdTomato mice expressed the calcium reporter GCaMP5g (yellow) and tdTomato (red), which were not detected in brain slices from noninduced animals (Left). Acute laser lesion (star in the transmitted light channel, T-PMT) evokes a rapid [Ca²⁺]ᵢ rise in tdTomato-labeled microglia (yellow arrowhead). Times indicate seconds from laser ablation. (B and C) Spontaneous Ca²⁺ transients in GCaMP5g-expressing microglia occur less often in cells treated with 50 μM bupivacaine (BV) or 50 μM TPA compared to control cells, as shown by the representative traces for GCaMP fluorescence changes (ΔF/F) (B) and transient rates over 5 min (C). (D) Representative microglial cells incubated in the absence (control) or presence of 50 μM BV or 50 μM TPA (ΔF/F for GCaMP5g shown at peak). (E) [Ca²⁺]ᵢ levels over time showing increase upon laser lesion (vertical dashed line). TPA (n = 13 from 3 mice; P = 10⁻⁴) and BV (n = 22 cells from 4 mice; P = 3 x 10⁻³) significantly reduced the lesion-induced [Ca²⁺]ᵢ rise compared to control (n = 56 from eight mice). No significant difference was found between BV and TPA (P = 0.2). (F) Representative single-plane images of microglia (Iba1, red) in acute hippocampal rat slices incubated with 3-μm microbeads (FITC, green) in the absence (control) or presence of 50 μM BAPTA-AM to chelate intracellular calcium. Black arrowheads indicate nonphagocytic cells, and white arrowheads indicate phagocytic cells. (G) Percentage of microglia that phagocytosed microbeads in each condition, showing a significant reduction by BAPTA-AM (eight slices from five animals per condition; animals were used as the statistical unit).
Fig. 3. Microglial phagocytosis of presynaptic material is reduced by THIK-1 KO. (A) Representative confocal images from the CA1 stratum radiatum of WT or THIK-1 KO mice at P17 to P19, showing the presynaptic marker Bassoon (green) and the excitatory postsynaptic marker Homer1 (red). The merged image and expanded views on the right show colocalized puncta (yellow). (B–D) Quantification of the area covered by (B) Bassoon, (C) Homer1, and (D) colocalized puncta, showing an increased fraction of the imaged area labeled for synapses in KO mice. (E–G) Quantification of the numbers of (E) Bassoon, (F) Homer, and (G) synaptic puncta per square millimeter, showing increased numbers of presynaptic puncta and synapses in KO mice. Average numbers in WT mice were 53 puncta/100 μm² for Bassoon, 42 puncta/100 μm² for Homer1, and 26 synapses/100 μm². (For B–G, WT: n = 4 animals; KO: n = 3 animals; and three confocal stacks from five brain slices averaged per animal.) (H) Representative confocal images showing Bassoon puncta (green) located within microglia (Iba1, red). On the right, close-up of microglial processes with orthogonal projections at the level of the crosshairs showing Bassoon puncta within microglia. (I) Quantification of the area of Bassoon puncta colocalizing with each microglial cell, showing a decrease in KO microglia (WT: 20 cells from four animals; KO: 15 cells from three animals; and animals were used as the statistical unit).
in brain slices (∼0.13 transients/min; Fig. 2 B and C). Blocking THIK-1 channels with 50 μM bupivacaine or 50 μM TPA significantly decreased the frequency of these events (Fig. 2 B and C). In addition, focal laser lesions were used as a well-established proxy to evoke Ca^{2+} responses in microglia. Lesions triggered a rapid Ca^{2+} rise (Fig. 2 D), in agreement with previous reports (53). While untreated microglia exhibited consistent evoked Ca^{2+} responses, block of THIK-1 currents reduced this response (Fig. 2 D and E).

Buffering of intracellular calcium concentration with 50 μM BAPTA-AM (a membrane-permeable ester form of BAPTA) reduced microglial phagocytic rate by 75% (Fig. 2 F and G), supporting the idea that Ca^{2+} is required for microglial phagocytosis. Thus, reduction of Ca^{2+} transient activity by THIK-1 may provide a mechanism to explain its role in microglial phagocytosis.

**THIK-1 Regulates Microglial Phagocytosis of Synapses.** Deficits in microglial phagocytosis could result in impaired pruning of synapses during development (54), so we tested the effects of THIK-1 KO on hippocampal synapse numbers. Using P17 to P19 mice, when synapse pruning in the hippocampus is near
its peak (12, 13), we assessed the labeling of presynaptic (Bas- 
osoon) and postsynaptic glutamatergic (Homer1) markers in the 
stratum radiatum of the CA1 hippocampal region by immunohistochemistry (33). A colocalization of both markers was 
taken to indicate an excitatory synapse (Fig. 3A). In the 
THIK-1 KO, while no postsynaptic change was detected, the 
fraction of the imaged area labeled by the presynaptic marker 
approximately doubled compared to that in wild-type litter-
mates (WT). As a result, the derived total synaptic area was 
higher in KO mice (Fig. 3B–D). The increase in colocalization 
was produced by a 67% increase in the number (Fig. 3E–G) 
but not the size (SI Appendix, Fig. S3) of presynaptic terminals, 
with no change in the number of postsynaptic terminals 
(Fig. 3F).

To demonstrate that the increase in presynaptic terminal 
number was caused by a reduction of phagocytosis, we next 
assessed the presence of Bassoon puncta inside Iba1-labeled 
microglia (Fig. 3H). In THIK-1 KO mice, Bassoon colocaliza-
tion with microglia was significantly reduced (Fig. 3I). By con-
trast, Homer1 colocalization with microglia was not altered in 
the KO (SI Appendix, Fig. S4 A and B) and the density of den-
dritic spines was not affected either (SI Appendix, Fig. S4 C 
and D). Taken together, these data suggest that THIK-1 regulates 
the number of glutamatergic synapses by promoting microglial 
uptake specifically of presynaptic material.

THIK-1 Regulates Excitatory Synaptic Transmission. To further con-
firm that THIK-1 regulates removal of functional excitatory 
synapses, we performed whole-cell voltage-clamp recording of 
CA1 pyramidal neurons from P17 to P19 mice (Fig. 4). We 
found that the spontaneous EPSC frequency was enhanced in 
pyramidal neurons from THIK-1 KO compared to WT mice, 
with no amplitude alteration (Fig. 4A–C). Since this increase 
in the KO could be due either to a higher number of excitatory 
synapses or to higher activity (or an increased vesicle release 
probability) of presynaptic neurons, we bath-applied TTX to 
block action potential–mediated neurotransmitter release. Con-
sistent with our immunohistochemical studies showing more 
synapses in the KO (Fig. 3), mEPSC frequency approximately 
doubled in THIK-1 KO pyramidal neurons compared to in WT 
cells, without an amplitude change (Fig. 4A, D, and E).

This effect of THIK-1 deletion on synapse levels did not 
result from an altered number of microglia, as overall micro-
glial density and distribution in CA1 were similar between 
THIK-1 WT and KO mice (SI Appendix, Fig. S5). Furthermore, 
we found that THIK-1 only regulates excitatory synapses, with 
no detectable effect on inhibitory synapses. Neither the fre-
cuencies nor amplitudes of sIPSCs or mIPSCs were affected by 
THIK-1 KO (SI Appendix, Fig. S6). Altogether, our data sug-
gest that THIK-1 deficiency leads to an increase in the number 
of functional excitatory synapses, which is due to THIK-1 regu-
larizing microglial internalization of excitatory presynaptic 
terminals, presumably via its effects on the microglial membrane 
potential and/or [Ca\(^{2+}\)] transients.

We tested postsynaptic effects of THIK-1 deficiency electrophysiologically (Fig. 4F and G) by bath-aplying the glutamate 
receptor agonists NMDA (to activate NMDA receptors, 10 
µM; Fig. 4F) and kainic acid (to activate AMPA/KA receptors, 
1 µM; Fig. 4G). Consistent with our staining showing no effect 
on postsynaptic terminals (Fig. 3C and SI Appendix, Fig. S4), 
we found no significant differences between the THIK-1 KO 
and WT in their NMDA- and kainate-induced macroscopic cur-
rents (Fig. 4 F and G). Thus, THIK-1 deficiency selectively 
enhances the number of excitatory presynaptic release sites 
without affecting the postsynaptic glutamate receptor density 
(assessed from the spontaneous and miniature EPSC ampli-
tudes) or the total (synaptic plus extrasynaptic) glutamate 
receptor density (assessed from the response to NMDA and 
kainate). Interestingly, the effect of THIK-1 deletion on excit-
atory synapse number was transient during development, as no changes in synapse number (SI Appendix, Fig. S7) or synaptic 
transmission (SI Appendix, Fig. S8) were detected between 
adult WT and KO mice. This supports the notion of a key 
developmental role for THIK-1-mediated regulation of synapse 
number, which may also become reactivated in disease.

Discussion

Microglia are not merely passive support cells. Instead, they 
continuously survey the brain parenchyma and control neuronal 
function. THIK-1 channels, the main K\(^+\) channels in “resting” 
microglia, regulate their ramification, surveillance, and cytokine 
release (3). Here, using pharmacology and THIK-1 KO mice, we 
demonstrated that microglial phagocytosis is also controlled 
by THIK-1 (Fig. 1).

The requirement of THIK-1 for phagocytosis may in part be 
due to its role in enhancing microglial ramification and surveil-
lanse, which will increase the probability of a microglial cell 
encountering a target to phagocytose. However, the tonically 
active THIK-1 may also promote phagocytosis by keeping 
microglia hyperpolarized (3), and this hyperpolarization will 
increase the driving force for Ca\(^{2+}\) entry. Indeed, a hyperpolar-
ized membrane voltage is associated with phagocytosis in 
macrophages (20), while depolarization is seen in TREM2 
KO microglia in which phagocytosis is reduced (21). Fur-
thermore, we found that THIK-1 block reduced spontaneous 
and damage-evoked Ca\(^{2+}\) transient activity in microglia (Fig. 2
A–E), which may be required for phagocytosis (41, 42, 44, 50), 
consistent with our demonstration that buffering [Ca\(^{2+}\)], 
reduced microglial phagocytosis (Fig. 2F and G). A need for 
calcium concentration changes in phagocytosis and the fact 
that THIK-1 KO reduces such changes provide a possible mecha-
nism by which THIK-1 KO inhibits phagocytosis. Protein levels 
of the microglial lysosome marker CD68 were also reduced in 
the THIK-1 KO (Fig. 1G and H). These data suggest that there 
is a specific effect of THIK-1 KO on phagocytosis and down-
stream lysosomal degradation, although we cannot rule out a 
contribution by the decreased surveillance in microglia lacking 
THIK-1.

THIK-1 is expressed both in microglia and oligodendrocytes 
(49). In the absence of a microglial-selective THIK-1 KO 
mouse being available, four independent results indicate that 
the effects of global THIK-1 KO on synapse number are medi-
ated by microglial changes. First, THIK-1 KO roughly halved 
the number of microglia which phagocytose fluorescent 
microbeads (Fig. 1E). Second, we show that THIK-1 KO 
reduced by 40% the amount of the presynaptic protein Bassoon 
that was internalized into microglia (Fig. 3H and J). Third, 
microglia are the only phagocytic cell type expressing THIK-1 
channels in the brain (49) as oligodendrocytes reportedly lack 
phagocytic machinery (55). Finally, the protein level of a micro-
glial lysosomal marker (CD68) was approximately halved in 
the THIK-1 KO hippocampus, while the level of a lysosomal 
marker (LAMP1) expressed by other brain cells (including 
astrocytes and oligodendrocytes) was unchanged (Fig. 1G–J). 
These data are consistent with a direct role for THIK-1 in 
controlling phagocytosis by microglia specifically, although indirect 
signaling to microglia via oligodendrocytes (where this channel 
is also expressed) cannot be excluded.

As a result of impaired phagocytic ability in THIK-1 KO 
microglia, the number of hippocampal excitatory synapses was 
increased in these mice during development, as shown both by 
immunolabeling and electrophysiology (Figs. 3 and 4). We 
found that THIK-1-mediated promotion of synapse loss was 
amainly an effect on the number of presynaptic terminals (Fig. 3
E–G). While others have reported internalization of postsynaptic
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material as well (13, 56), there is now evidence that microglial phagocytosis preferentially targets presynaptic compartments both in health (12, 14) and disease (25, 26, 57). This might be partly due to “eat-me” tags (such as C1q) (58) being preferentially located on presynaptic sites. Here, reported changes in excitatory synapse number (Fig. 3) and synaptic transmission (Fig. 4) reflect the net effect of THIK-1 on synapse formation and removal. While we directly assess engulfment of synaptic material by microglia (Fig. 3 and SI Appendix, Fig. S4), an additional contribution of THIK-1 to synapse formation—which may be induced by microglial contact (11, 12)—cannot be excluded.

On the other hand, we found that deleting THIK-1 had no effect on inhibitory synapses (SI Appendix, Fig. S6), suggesting that phagocytosis by microglia mainly targets excitatory synapses, and thus that there is a difference in the recognition molecules expressed on excitatory versus inhibitory synapses. Indeed, microglial depletion increases mEPSC frequency in brain slices (59) and in vivo (60), while mIPSCs remain unaltered (60).

In line with reports that microglial phagocytosis may affect synapse numbers transiently during development (7, 13), we found that THIK-1 deletion did not affect synapse numbers in healthy adult mice (SI Appendix, Fig. S7). This suggests that another mechanism operating in parallel can, on a long time scale, correct the number of synapses present. Nevertheless, developmental pruning mechanisms are activated again in disease scenarios after the normal developmental period is over, which can be detrimental if synapse removal is excessive (61).

In fact, synapse loss is a strong indicator of cognitive decline (62–64). Synaptic deficits precede amyloid deposition in animal models of dementia (65), and microglial phagocytosis of synaptic material is increased in Alzheimer’s patients (66). Ablation of microglia rescues synaptic loss and reduces memory impairment in mouse models of dementia (67). Thus, manipulating microglia–synapse interactions may provide clinical benefit for conditions causing cognitive impairment. Specifically, being able to block microglial phagocytosis in a time-controlled manner could help protect synapses from removal. As human microglia express Kcnk13, the gene encoding THIK-1 (68, 69), a role of microglial THIK-1 in regulating human synapse turnover is conceivable. It would be interesting to examine whether short-term block of THIK-1 in live human brain slices (e.g., using THIK-1-blocking anesthetics such as isoflurane or sevoflurane) increases synapse number, and the magnitude and duration of any such effect. Microglial responses (and especially phagocytosis) are crucial for the development and progression of dementia (70). Since amyloid-targeting therapies for Alzheimer’s disease have largely failed, possibly because therapeutic interventions are given too late (62), it would be advantageous to devise therapeutic agents that control phagocytosis to prevent synapse loss early on.

Data Availability. Code for analyzing microglial properties and calcium concentration changes data have been deposited in GitHub (https://github.com/AttwellLab). All other study data are included in the article and/or SI Appendix.

ACKNOWLEDGMENTS. This work was supported by European Research Council (BrainEnergy) and Wellcome Investigator Awards (099222) to D.A., a Wellcome Trust four-year PhD studentship to P.I., and a Chulabhorn Royal Academy PhD studentship to C.H. For the purpose of open access, the authors have applied a CC-BY public license to any author accepted manuscript version arising from this submission. We thank Sandra Alvarez for technical assistance and Anna Barkaway, Damian Cummings, Frances Edwards, Soyon Hong, Vasiliki Kyragyrji, Jonathan Lenzmy, Yuening Li, Christian Madray, Thomas Pfeiffer, Tania Quintela-López, Patricia Salinas, and James Scott-Solache for comments on the manuscript.
35. S. E. Haynes et al., The P2Y, receptor regulates microglial activation by extracellular nucleotides. Nat. Neurosci. 9, 1512–1519 (2006).
36. Z. I. Blume, J. M. Lambert, A. G. Lovel, D. M. Mitchell, Microglia in the developing retina: coupling phagocytosis with the progression of apoptosis via P2RY12 signaling. Dev. Dyn. 249, 723–740 (2020).
37. I. Diaz-Aparicio et al., Microglia actively remodels adult hippocampal neurogenesis through the phagocytosis-secretome. J. Neurosci. 40, 1453–1482 (2020).
38. C. J. Bohlen et al., Identification of requirements for microglial survival, specification, and function revealed by defined-medium cultures. Neuron 94, 759–773.e8 (2017).
39. O. Butovsky et al., Identification of a unique TGF-β-dependent molecular and functional signature in microglia. Nat. Neurosci. 17, 131–143 (2014).
40. Y. Mao, S. C. Finnemann, Regulation of phagocytosis by Rho GTPases. Biochim. Biophys. Acta 131, 1014–1024 (2011).
41. P. Nunes, N. Demaurex, The role of calcium signaling in phagocytosis. J. Leukoc. Biol. 88, 57–68 (2010).
42. E. Urbe-Qerol, C. Rosales, Phagocytosis: Our current understanding of a universal biological process. Front. Immunol. 11, 1066 (2020).
43. M. L. Albert et al., Immature dendritic cells phagocytose apoptotic cells via alphavbeta5 and CD36, and cross-present antigens to cytotoxic T lymphocytes. J. Exp. Med. 188, 1359–1368 (1998).
44. S. Koizumi et al., UDP-acting at P2Y receptors is a mediator of microglial phagocytosis. Nature 446, 1091–1095 (2007).
45. R. X. Wen et al., P2Y receptor inhibition aggravates ischemic brain injury by reducing microglial phagocytosis. CNS Neurosci. Ther. 26, 416–429 (2020).
46. R. Munoz-Planillo et al., K⁺ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. Immunity 38, 1142–1153 (2013).
47. D. P. Lotshaw, Biophysical, pharmacological, and functional characteristics of cloned and native mammalian two-pore domain K⁺ channels. Cell Biochem. Biophys. 47, 209–256 (2007).
48. P. L. Piechotta et al., The pore structure and gating mechanism of K₂p channels. EMBO J. 30, 3607–3619 (2011).
49. Y. Zhang et al., An RNA sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. J. Neurosci. 34, 11929–11947 (2014).
50. M. A. Gronski, J. M. Kinchen, I. J. Juncadella, N. C. Franc, K. S. Ravichandran, An essential role for calcium flux in phagocytes for apoptotic cell engulfment and the anti-inflammatory response. Cell Death Differ. 16, 1323–1331 (2009).
51. B. Brawek, O. Garaschuk, Microglial calcium signaling in the adult, aged and diseased brain. Cell Calcium 53, 159–169 (2013).
52. B. Brawek et al., Impairment of in vivo calcium signaling in amyloid plaque-associated microglia. Acta Neuropathol. 127, 495-505 (2014).
53. G. Eichhoff, B. Brawek, O. Garaschuk, Microglial calcium signal acts as a rapid sensor of single neuron damage in vivo. Biochim. Biophys. Acta 1813, 1014–1024 (2011).
54. D. P. Schafer, E. K. Lehman, B. Stevens, The “quad-partite” synapse: Microglia-synapse interactions in the developing and mature CNS. Glia 61, 24–36 (2013).
55. A. Brosius Lutz, B. A. Barres, Contrasting the glial response to axon injury in the central and peripheral nervous systems. Dev. Cell 28, 7–17 (2014).
56. G. O. Sipe et al., Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex. Nat. Commun. 7, 10905 (2016).
57. G. Gunner et al., Sensory lesioning induces microglial synapse elimination via ADAM10 and fractalkine signaling. Nat. Neurosci. 22, 1075–1088 (2019).
58. B. A. Gyorffy et al., Local apoptotic-like mechanisms underlie complement-mediated synaptic pruning. Proc. Natl. Acad. Sci. U.S.A. 115, 6303–6308 (2018).
59. K. Ji, G. Akgul, L. P. Wollmuth, S. E. Tsirka, Microglia actively regulate the number of functional synapses. PLoS One 8, e56293 (2013).
60. X. Ma et al., Depletion of microglia in developing cortical circuits reveals its critical role in glutamatergic synapse development, functional connectivity, and critical period plasticity. J. Neurosci. Res. 98, 1968–1986 (2020).
61. M. W. Salter, B. Stevens, Microglia emerge as central players in brain disease. Nat. Med. 23, 1018–1027 (2017).
62. J. M. Long, D. M. Holtzman, Alzheimer disease: An update on pathobiology and treatment strategies. Cell 179, 312–339 (2019).
63. R. D. Terry et al., Physical basis of cognitive alterations in Alzheimer’s disease: Synapse loss is the major correlate of cognitive impairment. Ann. Neurol. 30, 572–580 (1991).
64. S. T. DeKosky, S. W. Scheff, S. D. Styren, Structural correlates of cognition in dementia: Quantification and assessment of synapse change. Neurodegeneration 5, 417–421 (1996).
65. D. M. Cummings et al., First effects of rising amyloid-β in transgenic mouse brain: Synaptic transmission and gene expression. Brain 138, 1992–2004 (2015).
66. M. Tzioras et al., Altered synaptic ingestion by human microglia in Alzheimer’s disease. bioRxiv [Preprint] (2019). https://www.biorxiv.org/content/10.1101/795930v1 (Accessed 23 August 2021).
67. E. E. Spangenberg et al., Eliminating microglia in Alzheimer’s mice prevents neuronal loss without modulating amyloid-β pathology. Brain 139, 1265–1281 (2016).
68. Y. Zhang et al., Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse. Neuron 89, 37–53 (2016).
69. T. F. Gaitani et al., Transcriptomic analysis of purified human cortical microglia reveals age-associated changes. Nat. Neurosci. 20, 1162–1171 (2017).
70. A. Podlesi-Drabiniok, E. Marcara, A. M. Goate, Microglial phagocytosis: A disease-associated process emerging from Alzheimer’s disease genetics. Trends Neurosci. 43, 965–979 (2020).