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Microglial Contact Prevents Excess Depolarization and Rescues Neurons from Excitotoxicity

Microglia Rescue Neurons from Excitotoxic Insult

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Abstract

Microglia survey and directly contact neurons in both healthy and damaged brain but the mechanisms and functional consequences of these contacts are not yet fully elucidated. Combining two-photon imaging and patch-clamping, we have developed an acute experimental model for studying the role of microglia in CNS excitotoxicity induced by neuronal hyperactivity. Our model allows us to simultaneously examine the effects of repetitive supramaximal stimulation on axonal morphology, neuronal membrane potential, and microglial migration, using cortical brain slices from Iba-1 eGFP mice. We demonstrate that microglia exert an acute and highly localized neuroprotective action under conditions of neuronal hyperactivity. Evoking repetitive action potentials (APs) in individual layer 2/3 pyramidal neurons elicited swelling of axons, but not dendrites, which was accompanied by a large, sustained depolarization of soma membrane potential. Microglial processes migrated to these swollen axons in a mechanism involving both ATP and glutamate release via volume-activated anion channels. This migration was followed by intensive microglial wrapping of affected axons, and in some cases removal of axonal debris, that induced a rapid soma membrane repolarization back to resting potentials. When the microglial migration was pharmacologically blocked, the activity-induced depolarization continued until cell death ensued, demonstrating that the microglia-axon contact served to prevent pathological depolarization of the soma and

maintain neuronal viability. This is a novel aspect of microglia surveillance, detecting, wrapping and rescuing neuronal soma from damage due to excessive activity.

Significant Statement

Microglia, as immune cells in central nervous system, are highly motile cells, continuously expanding and retracting their processes as they monitor brain parenchyma. They can exert neuro-protective or neuro-toxic effects depending on their activation state. In this paper, we demonstrate that microglia are attracted to over-active axons, directly connecting to these axons to reduce membrane potential and exert neuro-protection. The attraction of microglia processes depends on ATP release through volume activated anion channels (VAACs). Blocking VAACs inhibited microglial attraction to axons and impaired the restoration of membrane potential and axonal survival.

Introduction

Microglia are the immune cells of the central nervous system (CNS), responding to disruptions of brain integrity by changing to a morphologically and biochemically distinct activated state which then plays an important role in inflammation and clearance of neuronal debris after cell death (Hanisch and Kettenmann 2007; Ransohoff and Perry 2009). Non-activated microglia are also emerging as important elements of neuronal homeostasis in the developing and healthy

brain, actively surveying the brain parenchyma and making frequent contacts with different components of neuronal circuitry (Wake et al. 2009). During development microglia can sculpt neuronal circuits by interactions and phagocytosis, via signaling mechanisms that may include fractalkine receptors (Paolicelli et al. 2011) and complement pathways (Stevens et al. 2007). These microglia-neuron contacts are more frequent and/or extensive in more active neurons (Wake et al. 2009). In larval zebrafish, such preferential microglial contacts with active neurons have been proposed to result in a selective reduction of neuronal activity (Li et al. 2012). Hence excessive neuronal activity, such as occurs during seizures, could be expected to acutely attract resting ramified microglia and potentially trigger their activation or functional consequences. Epileptic rodent and human brains are characterized by chronic microglial and astrocytic activation (Devinsky et al. 2013). The proliferation and activation of microglia in rodent brain begins within three hours following chemically induced status epilepticus, but develop more strongly over the subsequent 2 days (Avignone et al. 2008). Such microglial changes can occur in the absence of clear necrosis, and reducing this activation phenotype can reduce the subsequent loss of neurons associated with status epilepsy (Ulmann et al. 2013). Coupled with the ability of microglia-derived inflammatory mediators to potentially enhance excitability, this suggests a largely detrimental role of chronic microglial activation in sustaining seizures (Devinsky et al. 2013). However, microglia can also be protective in brain pathologies (Biber et

al. 2014) and, indeed, pre-conditioned activated microglia may in fact reduce seizure threshold (Mirrione et al. 2010) while non-activated ramified microglia can also be protective against excitotoxicity in cultured neuronal models (Vinet et al. 2012). Given that most studies correlate microglia and neuronal phenotypes during chronic or prolonged hyperexcitability or excitotoxic models, we focus here on acute interactions between microglia and hyperactive neurons, and the possible functional consequences of such interaction. We developed a novel cortical slice experimental model in which we could simultaneously measure neuronal membrane potential, axonal neuronal morphology and microglia dynamics. We show that microglia migrate to axons swollen by excessive activity, and can wrap and pinch off these affected axonal regions, thereby protecting neurons from possible excitotoxic depolarization.

Materials & Methods

All experimental procedures involving animals were approved by the National Institute for Physiological Sciences Animal Care and Use Committee and were in accordance with NIH guidelines. Coronal slice preparation, the shadow patch method (Kitamura et al. 2008), and two-photon imaging were used as described previously (Wake et al. 2009). Experiments were carried out in 250 μm coronal slices of S1 cortex from Iba1 eGFP mice (both sex 28-42 d, n=111) (Hirasawa et al. 2005). To avoid recording from neurons near microglia activated by the brain slicing, we used neurons usually at a depth below 70 μm from the slice surface. The

morphology of the microglia at this depth was comparable to that in intact brain (Stence et al., 2001). Furthermore, only a single slice in each mouse was used as microglia in subsequent slices seemed to show a gradual activation. The microglia we analyzed under these conditions were judged "non-active" consistent with previous studies (Avignone et al., 2015; Haynes et al., 2006) in that the number of processes were more than six and the area of the soma was less than 50 μm^2.

The Krebs' solution used for maintenance and recordings contained (in mM): 117 NaCl, 3.6 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, and 11 glucose, equilibrated with 95% O_2 and 5% CO_2. Drugs used were tetrodotoxin (Latoxan, Valence, France); 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX), (RS)-α-methyl-4-carboxyphenylglycine disodium salt (MCPG), and (RS)- α-methyl-4-sulfonophenylglycine (MSPG) (Tocris, St. Ellisville, MO, USA); and 5-nitro-2-(3-phenylpropylamino) benzonic acid (NPPB), pyridoxal phosphate-6-azo (benzene-2, 4-disulfonic acid (PPADS), suramin sodium salt, and DL-2-amino-5-phosphonovaleric acid (AP-V), (Sigma-Aldrich, St. Louis, MO, USA).

Dual-color fluorescence images were collected using a two-photon laser-scanning microscope (FV-1000 MPE, Olympus, Tokyo) fitted with a water-immersion 40X /0.80 numerical aperture objective lens (Olympus) that was coupled to a Ti-Sapphire laser (MaiTai Deep See, Spectra Physics, Santa Clara, CA, USA) tuned at the wavelength of 950 nm, which simultaneously

137 excited both Alexa-594 and eGFP. Time-lapse imaging was performed by repeated acquisition of
138 small fluorescence image stacks comprising 15-20 focal planes, each with 0.75 µm axial spacing,
139 and sets of images were obtained every 3 minutes. The spatial limitation of resolution of the
140 two-photon imaging was 1-2 µm in the X-Y plane, and 3-4 µm for the Z axis. Patch pipettes
141 (5-7 MΩ) were filled with (in mM) 136 K-gluconate, 5 TEA-Cl, 0.5 $CaCl_2$, 2 $MgCl_2$, 5 EGTA,
142 5 HEPES, 5 Mg ATP, and Alexa 594 hydrazide, sodium salt (Alexa 594; 80 µM) (Molecular
143 probes, Eugene, OR, USA). After obtaining the whole-cell configuration, 40-50 minutes was
144 allowed for diffusion of Alexa-594 into the processes. Depolarizing current-thresholds for APs
145 were determined using a series of 50-ms current pulses that increased in 50 pA increments. AP
146 trains were evoked using currents of 3 x threshold, 50-ms pulses, at 10 Hz. Membrane
147 conductance was determined in current clamp from the slope of voltage responses to current
148 steps from -200 pA to +120 pA (see Fig. 4). Measurements of axonal diameter as well as axonal
149 fluorescence intensity (FI) were made from time-lapse images acquired at 3-minute intervals,
150 from 18 minutes before until 24 minutes after the start of the 6-minute stimulus train. Relative
151 FI of axon and microglial processes during pre- and post-stimulus were quantified from the
152 averaged values over the 6 frames (18minutes) and 8 frames (24 minutes) respectively. Axonal
153 fluorescence intensity was strongly correlated with the square of axonal diameter (r=0.824,

p<.001), and so axonal FI was used as an indirect measure of changes in axonal cross-sectional area.

The regions of interest (ROIs) for measurement of fluorescence intensities were defined by tracing an axon or a dendrite and including the adjacent area within a lateral distance of 3 μm. Fluorescence intensities were defined as F/Fo (%)=[(F1-B1)/(F0-B0)] x 100, where F1 and F0 are the total ROI fluorescence at a given time and at the starting time of data acquisition (t=-18 min), respectively. B1 and B0 are the corresponding background FIs. Background values were taken from the darkest region of a uniformly illuminated field.

Experimental values are expressed as mean ± SEM. Student's t test was used for comparisons. Paired *t*-test was used as indicated. The difference in survival rates was estimated by Kaplan-Meier analysis.

Results

Volume changes in the axon induced by action potential firings and dynamic behavior of microglia in the peri-axonal area

To observe the dynamic behavior of microglial processes adjacent to an activated axon, we used the whole-cell patch-clamp technique to load single layer 2/3 pyramidal neurons with Alexa 594 dye in primary somatosensory (S1) cortex slices from Iba-1 eGFP mouse, and evoked trains of APs by current injection in the same neurons. Since the vast majority of microglia near the cut

surface of the slice were in an activated phenotype, we used shadow patch clamp technique (Kitamura et al. 2008) for the recording from deeper position within the slice (depth from the surface = 95.8 ±1.2 µm, n=133, Fig. 1A). A 6-minute train of APs evoked at 10Hz induced axonal swelling detected by an increase in fluorescence intensity (FI) of the red axonal Alexa 594 (increase of 5.95 ± 1.34%, $p<0.001$; see also Materials and Methods) (Fig. 1B-E & 2A). Axonal FI increases were typically accompanied by (green) FI increases resulting from recruitment of GFP-positive microglial processes (increase of 9 ± 3.42%, $p<0.05$) (Fig. 1B, 1G, 1H & 2A, Movie 1) which occurred with a delay of 0 -15 minutes (6.25 ± 1.40 minutes) following the axonal FI increase (Fig. 1D vs 1G, also see Fig. 3E). No microglial soma migration was observed. Given that somatic APs can back-propagate into dendrites in cortical neurons (Stuart and Sakmann 1994), a similar activity-induced swelling and microglial attraction may be predicted to occur in dendrites. However, the FI of apical and basal dendrites, and the adjacent peri-dendritic microglial FI, did not increase (Fig. 1D and 1G), suggesting microglia processes do not migrate on to active dendrites (Movie 2).

Longer trains of APs (10 Hz, 0.33~9 minutes) produced larger increases in both axonal and peri-axonal microglial FI (Fig. 1E & 1H), and these increases were significantly correlated ($r=0.644$, $p<0.001$) (Fig. 1F). Both increases were blocked by TTX (1 µM) (Fig. 2B), demonstrating that they were dependent on AP generation.

Involvement of VAACs in migration of microglial processes to swollen axons

Neuronal swelling activates volume activated anion channels (VAACs) that can release non-exocytotic ATP (Sabirov and Okada 2005), including from axons swollen by APs (Fields and Ni 2010). ATP is known to be a potent attractant of microglia (Davalos et al. 2005; Haynes et al. 2006; Ohsawa et al. 2007) through the P2Y receptor, thus we examined a potential role of VAACs and ATP release in microglial migration to swollen axons using pharmacological blockade (Fig. 2C & 2D). Migration of microglial processes to peri-axonal regions was absent in the presence of the VAAC blocker NPPB, although AP-induced axonal swelling persisted (Fig. 2C). A mixture of P2-purinegic receptor blockers, suramin and PPADS, also blocked the migration of microglial processes (pre-, 99.6 ± 0.397% vs post-, 101 ± 2.60%, p=0.560) without affecting the increase in axonal volume (Fig. 2D). Thus, ATP released via activation of VAACs appears to largely mediate the attraction of microglial processes.

VAACs also function as a pathway for the release of excitatory amino acids (Okada et al., 2009), and such excitatory amino acids has been both indirectly (Li et al., 2007; Fontainhas et al., 2011) and directly (Liu et al., 2009) reported to increase microglial process motility. Carbenoxolone also failed to block the axonal volume increase and migration of microglial processes to axons (Fig. 2E), suggesting that activation of gap junction hemichannels (Li et al.

2012) were not key contributors to the release of chemoattractant induced by axonal volume increase.

Microglial wrapping of damaged axons rapidly reverses the pathological depolarizations of neurons.

In a subset of neurons (7/38, 18%), the stimulation-induced axonal swelling was associated with large (> 20 mV) and rapid depolarizations of the soma membrane potential (V_m, Fig. 3B). Reversal of the depolarization was observed in every case following the migration and wrapping of microglial processes around the swollen axon (Fig. 3A-D). Some neurons had an additional cycle of depolarization and repolarization, in association with an additional episode of axonal swelling and microglial wrapping (e.g. Fig. 3B). To clarify the temporal relationship between the changes in axonal volume, V_m fluctuations and microglial processes migration, we compared (1) the onset of the increases in axonal and microglial FI and pathological V_m depolarizations and (2) the times when these three values returned to their baseline levels. Pooled data suggested that the increase in microglial FI never preceded the axonal FI increase nor the depolarization (Fig. 3C, n=7). Similarly, depolarization never preceded axonal FI increases. Complete retraction of microglial processes away from the axon as reflected in the return of peri-axonal microglial FI to baseline (change of <5%) did not occur until after the recovery of the V_m to the resting values (Fig. 3D, n=7). Together, the temporal relationships are

consistent with axonal swelling triggering both V_m depolarization and microglial process accumulation, while V_m recovery occurs during the microglial contacts. From these results, we proposed that, in response to excessively swollen axons, microglia make intimate contacts or wrapping of axons to "rescue" the neuronal somas from further progressive depolarizations. This rescue could be observed in response to accumulation of microglial processes alone (Fig. 3A and 3B). The repolarization induced by microglial wrapping could be due to changes in ionic current flowing across the axonal or soma membrane. In three neurons to which current steps were applied to examine the change in the membrane conductance during depolarization and after repolarization (Fig. 4A, B). The membrane conductance was significantly increased during depolarization and reduced after the sequential repolarization (Fig. 4C, D). This suggests channels or leak mediating inward current close upon the membrane repolarization, or that the soma becomes electrically uncoupled from regions of the affected axons.

Finally, we compared V_m depolarizations with and without NPPB, which blocks the microglial – axon interactions (Fig. 1J, 1M, 5A, 5B). The averaged data showed that following a long 6-minute 10Hz AP stimulus, a larger mean V_m depolarization was observed in the presence of NPPB (Fig. 5C, n=16 for control and n=8 for NPPB), which progressed to the complete loss

of the membrane potential ($V_m \approx 0$ mV, Fig. 5B) and resulted in acceleration of neuronal death with NPPB (Fig. 5D, $p<0.05$).

Discussion

We have developed an acute experimental model for studying the role of microglia in CNS excitotoxicity that allows us to simultaneously examine excitotoxic effects on axonal morphology, neuronal membrane potential, and microglial migration induced by suprathreshold stimulation of individual S1 cortical layer 2/3 pyramidal neurons. We used this model to directly demonstrate that microglia exert an acute and highly localized neuroprotective action under conditions of neuronal hyperactivity. Evoking of repetitive APs in individual pyramidal neurons resulted in selective swelling of axons, but not dendrites, which was accompanied in some cases by a large and sustained depolarization of somatic membrane potential. Microglial processes migrated to these swollen axons in a mechanism involving glutamate and ATP release via VAACs. This migration to swollen axons was followed by intensive microglial wrapping that induced a rapid somatic membrane repolarization back to its resting value. When the microglial migration was pharmacologically blocked, the activity-induced depolarization continued until cell death ensued, demonstrating that microglia-axon contact served to prevent pathological depolarization of the soma and maintain neuronal viability.

VAACs are ubiquitously expressed and activated by swelling, playing an important role in regulatory volume decreases in swollen cells (Okada et al. 2009). The release of ATP through axonal VAACs activated by swelling during trains of APs has been directly demonstrated in cultured dorsal root ganglion neurons (Fields and Ni 2010). Both P2X and P2Y ATP receptors are expressed on microglia (Xiang and Burnstock 2005) and ATP induces microglial processes migration towards the source of ATP (Davalos et al. 2005; Wu et al. 2007). Our result that NPPB and P2-purinergic receptor antagonists both block axonal swelling-induced microglial migration supports the idea that VAACs are activated by AP-induced swelling and release ATP to induce microglia chemotaxis to swollen axons. Given that VAACs are also permeable to glutamate (Liu et al. 2006) and microglia express a variety of glutamate receptors (Murugan et al. 2013), glutamate may additionally contribute to microglial-axon interactions. Excitotoxicity typically involves necrotic neuronal damage or death in conjunction with disruptions in cell volume and its regulation (Okada et al. 2009). In such models, microglia can be either neurotoxic (Biber et al. 2014; Cai et al. 2006; Yrjanheikki et al. 1998) or neuroprotective (Biber et al. 2014; Hulse et al. 2008; Neumann et al. 2006). However, these studies have assessed neuronal viability, histological damage and/or behavioral parameters at least a day after the onset of the damaging stimuli. The acute early microglial responses observed here, including engulfment of axons, may offer protection via physical

shielding of leaky axonal membranes, as proposed for leaky cerebral vessels (Nimmerjahn et al. 2005) by releasing neuroprotective signaling molecules (Kettenmann et al. 2011) and/or by phagocytozing damaged portions of axons to minimize the release of cytotoxic intra-axonal contents (Neumann et al. 2006; Streit 2002). the decreased membrane conductance observed during the axonal re-polarization indicates less permeability to ions with a more depolarized equilibrium potential, either through selective closure of channels or by electrical uncoupling from leaky axons (e.g., by sealing membrane damage). The functional consequences of this microglial response resemble a first-aid approach whereby they "bandage" or "excise" the damaged axons. The microglial first-aid may also function as a "circuit breaker" that prevents overexcitation of neurons post-synaptic to the hyperactive axons. Resting microglia can physically interact with neuronal elements in an activity-dependent manner in healthy brain (Wake et al. 2009), and these interactions reduce hyperactivity of neurons (Li et al. 2012). Our results reveal another facet to the mechanisms by which microglia detect abnormal neuronal structure or function and respond to maintain neuronal circuit homeostasis.

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Figure legends

Figure 1. Action potential (AP)-induced axonal swelling and subsequent migration of microglial processes to the peri-axonal area.

(A) A low magnification image of a cortical slice obtained from an Iba1-GFP mice in which microglia express GFP (green). Note the largely ramified morphology suggesting a "resting state", except for some surface microglia. A recording pipette and a pyramidal neuron from which a recording was obtained are loaded with Alexa594 (red). Scale bar, 50 µm. (B) Time-lapse images of a layer2/3 somatosensory cortical neuron axon filled with Alexa 594 (red) and surrounding microglial processes (expressing eGFP, green), acquired at different times before and after a 3-minute current stimulation protocol applied to the neuron's soma to evoke repetitive APs (at 10Hz). Scale bar, 3 µm. (C) Higher magnification images of axonal fluorescence signals from the areas indicated by the rectangular boxes at -12 minutes and 6 minutes in (B). Note the increase in fluorescent intensity (FI). Scale bar, 1 µm. (D and G) Mean change in relative axonal and microglial FIs; before, during and after 6-minutes of 10 Hz soma current stimulation applied from t=0 (n=16). Plots of the relative neuronal (red) FI (D) and peri-neuronal (green) microglial FI (G) for axons (including no stim controls) and dendritic compartments are superimposed (apical dendrite, black; basal dendrite, gray). (E and H) post-stimulus change in relative axonal (H) and microglial (H) FIs for different durations of stimuli. (F) Pooled data showing the correlation between the relative stimulus-induced changes in axonal FI and microglial FI obtained from a range of 10Hz stimulus train durations as indicated by each symbol.

Figure 2. Investigating roles of VAACs in action potential (AP)-induced axonal swelling and subsequent migration of microglial processes to the peri-axonal area.

(A-E) Plots of the pre- and post-stimulus data points for axonal swelling (red) and peri-axonal microglial (green) FI, for the control condition (A) and for each of the applied drugs (B-E). Individual, paired data and mean values (± SEM) are shown. FI was averaged over the pre- and post-stimulus time points, and these were compared using paired t-test. *p<0.05, **p<0.01, ***p<0.001.

Figure 3. Microglial wrapping of an axon and rescuing the depolarized somatic membrane potential.

(A) Time-lapse images of an axon (red) and surrounding microglial processes (green) before (i) and at various times as indicated (ii-vi) after a 6-minute period of supramaximal AP stimulation. Scale bar, 5 µm. Arrows at (iii) and (v) indicate extensive microglial accumulation around axon (B) Membrane potential (V_m, upper panel) and changes in relative axonal (red) and peri-axonal microglia (green) FI (lower panels) obtained from the experiment shown in (A). (i)-(vi) correspond to times shown in (A). Note that the Vm and FI are shown on the same timescale to illustrate the temporal associations between V_m depolarization, axonal swellings and the extensive accumulation of microglial processes around the axons that precedes repolarization and reduced swelling. (C, D) The temporal relationships between changes in axonal volume, membrane potential and the fluorescence intensity of peri-axonal microglial processes. The graphs plot data for each neuron of (C) the relative times of Vm depolarization, and how this relates to increased axonal swelling and microglial FIs, and (D) how the recovery of V_m relates to the recovery of axonal swelling (upper) and microglia FI (lower). In C and D, each colored line (with associated points) was obtained from a single experiment (n=7). Criterion for FI increase was >5% from the pre-stimulus intensity, and for depolarization was > 20mV from resting Vm. Vm recovery (time=0 in D) was defined as the return to within 10 mV of the initial

resting Vm, and microglial FI recovery (process retraction) was defined as the return to within 5% of the initial value.

Figure 4. Repolarization of membrane potential is associated with a decreased membrane conductance.

(A) Sample trace of membrane potential (V_m) before, during and after the application of a 6 minutes strong depolarizing stimulus. The regular positive and negative deflections reflect voltage responses to steps of current used to measure membrane conductance. labels (i) – (iii) indicate periods before, during and after the marked spontaneous, transient depolarization. (B) Current pulses (upper) and corresponding membrane potential responses (bottom) as used to assess passive membrane properties. Current steps ranged from -200 to +120 pA in 20-pA increments. (C) Representative membrane potential responses evoked by current pulses before the spontaneous depolarization (i), during the depolarization (ii) and after the depolarization (iii). Red dashed lines indicate the slope of the current –passive voltage relationship used to derive the membrane conductance. Note the action potentials in (i) evoked at more depolarized potentials. (D) Relative membrane conductance before during and after the sustained depolarization derived from fitting a linear regression to the relationship between applied current and subsequent voltage responses (as shown in C) to quantify the slope conductance ($\Delta I/\Delta V_m$). Circles indicate the mean values. Individual values are shown alongside means and SEM. Means were compared using a paired t-test. *$p<0.05$

Figure 5. Inhibition of microglial migration to swollen axons by block of VAACs.

(A) Time-lapse representative images of an axon (red) and microglial processes (green) at different times before and after a strong (6 mins) depolarizing stimulus, all in the presence of

VAAC block (Scale bar, 5 µm). (B) Representative traces of V_m and axonal and microglial FIs in the presence of NPPB, from a different neuron as shown in A. A sudden, large depolarization followed the stimulation-induced increase in axonal FI (red), but no increase in microglial FI (green) was seen adjacent to the axon, and no recovery of the depolarized V_m was seen (V_m continued to further depolarize to 0mV). (C) Pooled data showing he mean change of V_m following 6-minutes of AP stimulation, in control conditions (n=16, solid line) and in the presence of NPPB (n=8, dashed line). ***<0.005. (D) Kaplan Meier survival curves of neurons treated with NPPB (n=8, dashed line) and in control (n=16). *p<0.05. Neuronal "death" was defined as a neuron whose Vm depolarized to close to 0mV for more than a few minutes (typically followed by apparent loss of the Giga seal).

Movie 1. Time-lapse video showing an example of an excessively stimulated axon with a substantial volume increase, that was followed by intense contacts with microglial processes.

Note the swollen axon being repeatedly wrapped by microglial processes, after which the substantial membrane potential depolarizations were restored to the resting levels (See figure 4 for details). XY-projection images were acquired every 3 mins for 78 mins.

Movie 2. Time-lapse video showing microglial processes migrating to an axon but not to the dendrite.

The axon (bottom) was wrapped by microglial processes shortly after a substantial membrane potential depolarization was recorded. The neighboring basal dendrite (upper) was not wrapped by microglia during or after this depolarization. XY-projection images were acquired every 3 mins for 60 mins.