Resting Microglial Motility Is Independent of Synaptic Plasticity in Mammalian Brain

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Report

Resting Microglial Motility Is Independent of Synaptic Plasticity in Mammalian Brain

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Wu L-J, Zhuo M. Resting microglial motility is independent of synaptic plasticity in mammalian brain. *J Neurophysiol* 99: 2026–2032, 2008. First published February 6, 2008; doi:10.1152/jn.01210.2007. Microglia are well known for their roles in brain injuries and infections. However, there is no function attributes to resting microglia thus far. Here we performed a combination of simultaneous electrophysiology and time-lapse confocal imaging in green fluorescent protein–labeled microglia in acute hippocampal slices. In contrast to CA1 neurons, microglia showed no spontaneous or evoked synaptic currents. Neither glutamate- nor GABA-induced current/chemotaxis of microglia was detected. Strong tetanic stimulation of Schaffer-collateral pathways that induce CA1 long-term potentiation did not affect microglial motilities. Our results suggest that microglia are highly reserved for neuronal protective function but not synaptic plasticity in the brain.

INTRODUCTION

Microglia are the resident macrophages in the brain. Resting microglia rapidly transform into an activated state in most pathological processes, including host defense against infectious organisms, autoimmune inflammation, ischemia, trauma, neurodegeneration, and chronic pain (Hickey 2001; Streit et al. 2004; Tsuda et al. 2005). Activation of microglia is accompanied by changes in morphology, upregulation of immune surface antigens, and production of cytotoxic or neurotrophic molecules (Bruce-Keller 1999; Gonzalez-Scarano and Baltuch 1999; Kreutzberg 1996). In contrast, less is known about the function of resting microglia under physiological conditions in the brain. Recently, it was found that resting microglia have highly dynamic processes and survey the microenvironment in the brain in vivo (Davalos et al. 2005; Nimmerjahn et al. 2005) or in acute brain slices in vitro (Wu et al. 2007b). However, whether microglial motility is regulated or influenced by physiological neuronal functions, such as synaptic plasticity, remains mysterious.

Activity-dependent synaptic plasticity is thought to be important for memory formation and storage in the brain (Kandel 2001; Silva 2003). Hippocampal CA1 area is one of the most studied central regions for synaptic plasticity, such as long-term potentiation (LTP) (Bliss and Collingridge 1993; Malenka and Bear 2004). It was reported that attenuations in hippocampal LTP are associated with microglial activation in brain inflammation and infection (Griffin et al. 2006; Hauss-Wegrzyniak et al. 2002; Wang et al. 2004; Xiong et al. 1999).

However, whether LTP induction will affect microglial activities is unknown. To address this question, we performed simultaneous time lapse confocal imaging and electrophysiology of microglia in hippocampal CA1 region in mice expressing green fluorescent protein (GFP) exclusively in microglia. We found that resting microglia did not respond to neuronal activities, such as glutamatergic or GABAergic transmission, and microglia motilities are not altered in activity-dependent synaptic plasticity. Therefore our results provide the first evidence that microglia play a minor role in neuronal activity and plasticity.

METHODS

Transgenic mice

Heterozygous $Cx3cr1^{GFP/+}$ mice were used for all the experiments (Jung et al. 2000). All mice were maintained on a 12-h light/dark cycle with food and water provided ad libitum. All protocols used were approved by The Animal Care and Use Committee at the University of Toronto.

Acute brain slice preparation

Adult male or female mice (6–8 wk old) were anesthetized with 1–2% halothane and decapitated. Coronal brain slices (300 μm) containing hippocampus were prepared (Wu et al. 2007a) and transferred to a submerged recovery chamber with oxygenated (95% O_2-5% CO_2) artificial cerebrospinal fluid (ACSF) solution containing (in mM) 124 NaCl, 25 NaHCO$_3$, 2.5 KCl, 1 KH$_2$PO$_4$, 2 CaCl$_2$, 2 MgSO$_4$, and 10 glucose at room temperature. Microglia in brain slices were reported to be activated within several hours (Stence et al. 2001). To avoid the conversion from ramified to amoeboid microglia, all experiments were performed within 3 h after mice were killed.

Slice electrophysiology

Brain slices were transferred to a recording chamber and perfused with oxygenated ACSF solution at 3–4 ml/min at room temperature. Whole cell patch-clamp recordings were made on the soma of CA1 pyramidal neurons or GFP-labeled microglia. Recording electrodes (4–5 MΩ) contained an K$^+$-based internal solution composed of (in mM) 120 K-gluconate, 5 NaCl, 1 MgCl$_2$, 0.5 EGTA, 0.1 Na$_3$GTP, and 10 HEPES (pH 7.2; 280–300 mOsmol). Unless otherwise stated, the membrane potential was held at -60 mV for neurons and -20 mV for microglia throughout all experiments. For field recordings, a bipolar tungsten-stimulating electrode was placed in the stratum radiatum in the CA1 region, and extracellular field potentials were also recorded in the stratum radiatum using a glass microelectrode (4–5 MΩ) filled

with ACSF. The distance between stimulating and recording electrodes is ~100 μm. Stimulus intensity was adjusted to produce a response of ~0.5 mV amplitude. Test responses were elicited at 0.02 Hz. LTP was induced using two tetanic train stimulations (100-Hz, 1-s trains at a 20-s interval). Data were amplified and filtered at 2 kHz by a patch-clamp amplifier (Axopatch 200B), digitalized (DIGIDATA 1322A), stored, and analyzed by pCLAMP (Molecular Devices, Union City, CA). Data were discarded when the input resistance changed >20% during recording.

Confocal imaging

GFP-labeled microglia were imaged by confocal microscope (Fluoview FV 1000, Olympus, Tokyo, Japan). The laser with a wavelength of 488 nm was used for GFP excitation, and 633 nm was used for Alexa fluor 633 or DIC images. The image of microglia was collected for 8–10 consecutive focal steps of 2 μm once every 1 or 2 min using $\times$40, 0.8 numeric aperture water-immersion objectives. XYZT image galleries were collected, and Z projections were made for the quantification. To study the effect of tetanic stimulation on microglial motilities, we imaged one microglia from each slice. The position of microglia is between stimulating electrode and recording electrode, ~100 μm from the CA1 pyramidal cellular layer in the hippocampus.

Drug application procedures

All drugs were obtained from Sigma (St. Louis, MO). A picopump (pneumatic picopump, WPI, Sarasota, FL) was used to apply agonists to induce microglial chemotaxis and whole cell currents in microglia and neurons (Wu et al. 2007b). The diameter of the drug application pipette tip was ~3–4 μm. The pressure and duration of the puff was 5 psi and 100 ms, respectively.

Data analysis

The microglial motilities were analyzed by using Image Pro image analysis program. The number, distance, and volume of extending and retracting microglial processes were calculated every 2 min. Data were expressed as mean ± SE. Statistical comparisons were performed with the Student's t-test. In all cases, $P < 0.05$ was considered statistically significant.

RESULTS

Transgenic mice with GFP-labeled microglia provide a unique opportunity to study microglia in situ (Davalos et al. 2005; Jung et al. 2000; Nimmerjahn et al. 2005; Wu et al. 2007b). Freshly prepared hippocampal slices were used to characterize electrophysiological properties of microglia. Similar to what we reported in cortex and amygdala (Wu et al. 2007b), microglia are distributed evenly in the hippocampal slices. The average distance of microglial cell body in the hippocampus (61.3 ± 8.6 μm, $n = 9$ slices from 8 mice). Microglial cells in acute hippocampal slices showed highly
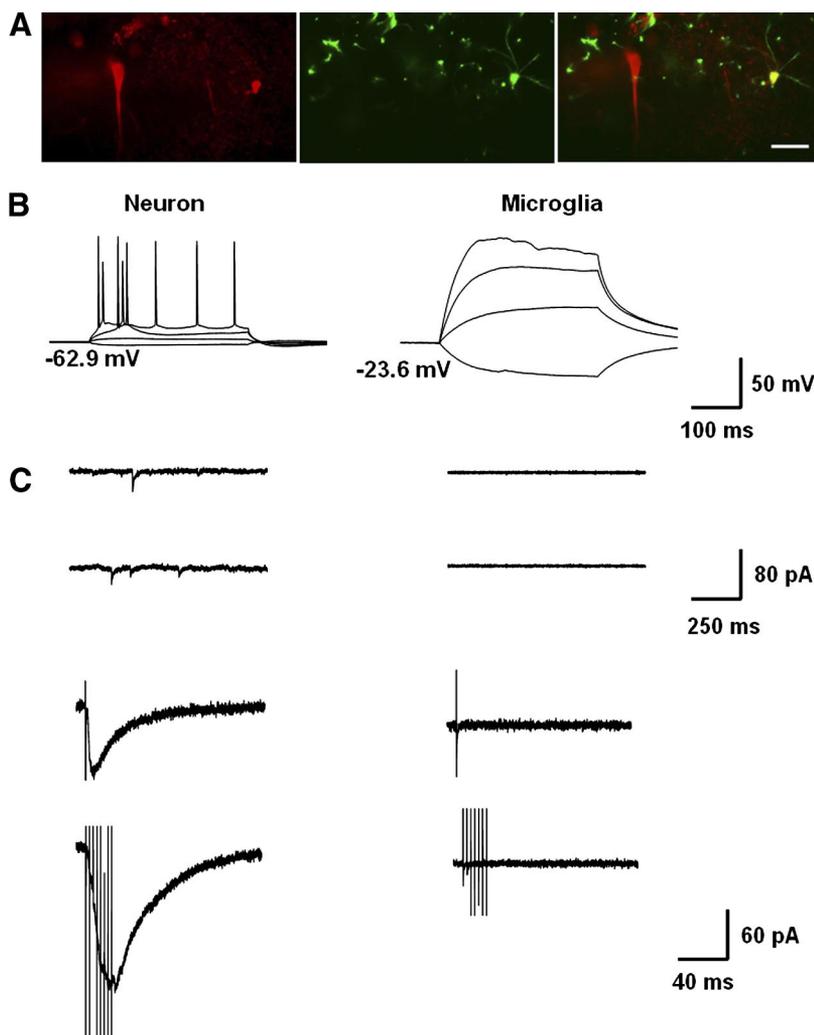

FIG. 1. No synaptic response was observed in resting microglia in the hippocampus. *A*: representative images showing whole cell recording in CA1 pyramidal neurons and nearby microglia. Recording cells were labeled in red color by intracellular infusion of Alexa fluor 633 (*left*). Microglia were labeled by green fluorescent protein (GFP) and shown in green (*middle*). Merged image confirmed the recording in GFP-labeled microglia (*right*). Scale bar, 22 μm. *B*: sample traces showing action potential was generated CA1 pyramidal neurons but not in microglia. Under current-clamp configuration, currents were injected (400-ms, 40-pA step) from −20 to 100 pA into neuron (*left*) or microglia (*right*). Action potentials were fired in neurons but not in microglia. However, microglia showed larger changes in membrane potentials responding to current injections. *C*: sample traces showing spontaneous synaptic currents were observed in neurons (*left*) but not in microglia (*right*). *D*: synaptic currents were evoked in neurons (*left*) but not in microglia (*right*). Stimulation of Schaffer collateral induced inward current in neurons (*top left*) and high-frequency stimulation (HFS; 100 Hz, 7 pulses) facilitated the current (*bottom left*). However, neither single stimulation nor HFS evoked any current in microglia.

ramified and motile processes, indicating they are mostly in resting states.

To study electrophysiological properties of microglia, we performed whole cell patch-clamp recordings in ramified microglia visualized by GFP-labeling in CA1 layer or in the stratum radiatum close to the CA1 layer in acute hippocampal slices (Fig. 1*A*). Nearby CA1 pyramidal neurons were also recorded for comparisons. A fluorescent dye, Alexa fluor 633, was used to further identify the neuronal and microglial morphology (Fig. 1*A*). In addition to the difference in morphology, all microglial cells showed smaller membrane capacitance (30.4 ± 1.1 pF, $n = 12$), higher membrane resistance (1.3 ± 0.2 GΩ, $n = 12$), and a more positive membrane potential (-22.5 ± 2.6 mV, $n = 12$). Under current-clamp configuration, injection of current induced larger changes in membrane potential in microglia, which is caused by their high membrane resistance. However, no action potential was generated even when 100 pA was injected ($n = 8$; Fig. 1*B*). The same amount of current injection caused action potential firing in all pyramidal neurons tested ($n = 6$).

A variety of neurotransmitter receptors, such as non-*N*-methyl-D-aspartate (NMDA) receptors, GABA$_B$ receptors, dopamine receptors, adrenergic receptors, and ATP receptors were reported to be expressed in cultured microglia in vitro (Farber and Kettenmann 2005; Fields and Burnstock 2006). To study whether microglia respond to neuronal activities, we recorded spontaneous and evoked synaptic currents at a holding potential of -60 mV under voltage-clamp configuration in neurons or microglia. No any antagonist was included in the ACSF. Spontaneous inward currents, which are probably mediated by non-NMDA receptors, were observed in CA1 pyramidal neurons ($n = 6$). In contrast, no spontaneous event was found in microglial cells ($n = 8$; Fig. 1*C*). Similarly, evoked inward currents were always induced in neurons ($n = 6$) but not microglia by a bipolar tungsten-stimulating electrode placed in the stratum radiatum (Fig. 1*D*). High-frequency stimulation (HFS; 100 Hz, 7 pulses) facilitated inward currents in neurons ($n = 6$). However, no current was observed in microglia by HFS ($n = 8$; Fig. 1*D*). In addition, HFS induced no current in microglia at a holding potential of -20 mV ($n = 8$).
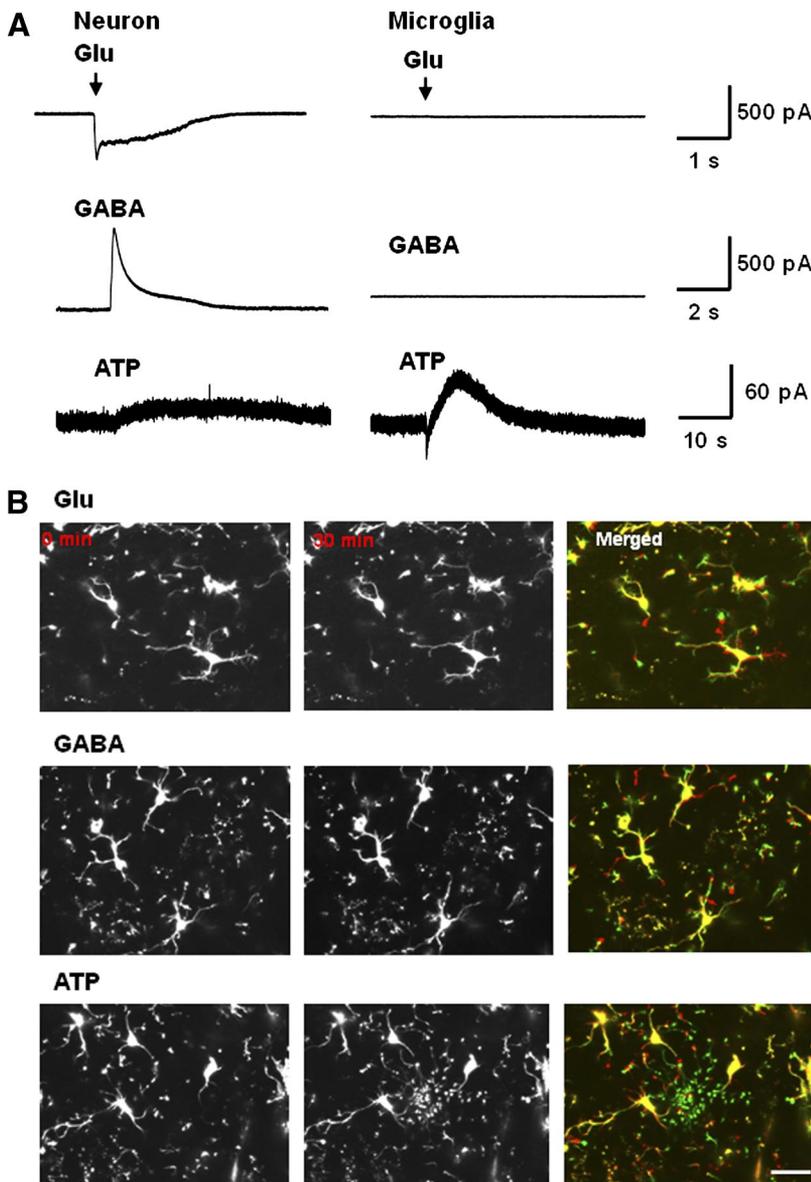

FIG. 2. Resting microglia did not respond to glutamate or GABA in the hippocampus. *A*: representative traces showing whole cell currents in CA1 pyramidal neurons (*left*) and nearby microglia (*right*). Local application of glutamate (Glu, 1 mM) induced inward currents in neurons but not in microglia (*top*), whereas GABA (1 mM) induced outward currents in neurons but no current in microglia (*middle*). However, ATP (1 mM) induced currents in both neurons and microglia (*bottom*). Microglia exhibited larger responses to ATP than neurons. Glutamate responses were recording at a holding potential of -60 mV. GABA and ATP response were recorded at a holding potential of -20 mV. *B*: sample images showing that ATP but not Glu or GABA could induce microglial chemotaxis. Glu (1 mM), GABA (1 mM), or ATP (1 mM) was locally applied in the center of the field, and microglial chemotaxis was imaged every 1 min for 30 min. The first image (0 min, *left*) and last image (30 min, *middle*) were shown here. To observe clearer chemotaxis, the 2 images were merged (*left*) and set as red and green for 0 and 30 min, respectively. Therefore the red reflects retracted portions, and the green reflects extended portions, whereas the yellow reflects unaltered portions. Local application of ATP induced dramatic microglia chemotaxis toward the pipette (*bottom*). However, neither Glu (*top*) nor GABA (*middle*) caused obvious microglial chemotaxis. Scale bar, 20 μm.

Taken together, these results indicate that microglial cells show no electrophysiological responses to neuronal activities under physiological conditions.

To further confirm the results, whole cell currents induced by local application of receptor agonists were studied in neurons and microglia (Fig. 2A). At a holding potential of −60 mV, local application of glutamate (1 mM) induced large inward currents, probably mediated by non-NMDA receptors, in neurons ($n = 6$) but there was no observable current in microglia cells ($n = 6$; Fig. 2A). At a holding potential of −20 mV, local application of GABA (1 mM) induced outward currents, probably mediated by GABA$_A$ receptors, in pyramidal neurons ($n = 6$) but not in microglia ($n = 6$; Fig. 2A). Therefore resting microglia did not respond electrically to major excitatory neurotransmitter, glutamate, or inhibitory neurotransmitter, GABA, in acute brain slices. We further studied ATP responses, which are important for the communication between neurons and microglia (Fields and Burnstock 2006). Similar to what we found in our previous study (Wu et al. 2007b), local application of ATP (1 mM) induced both
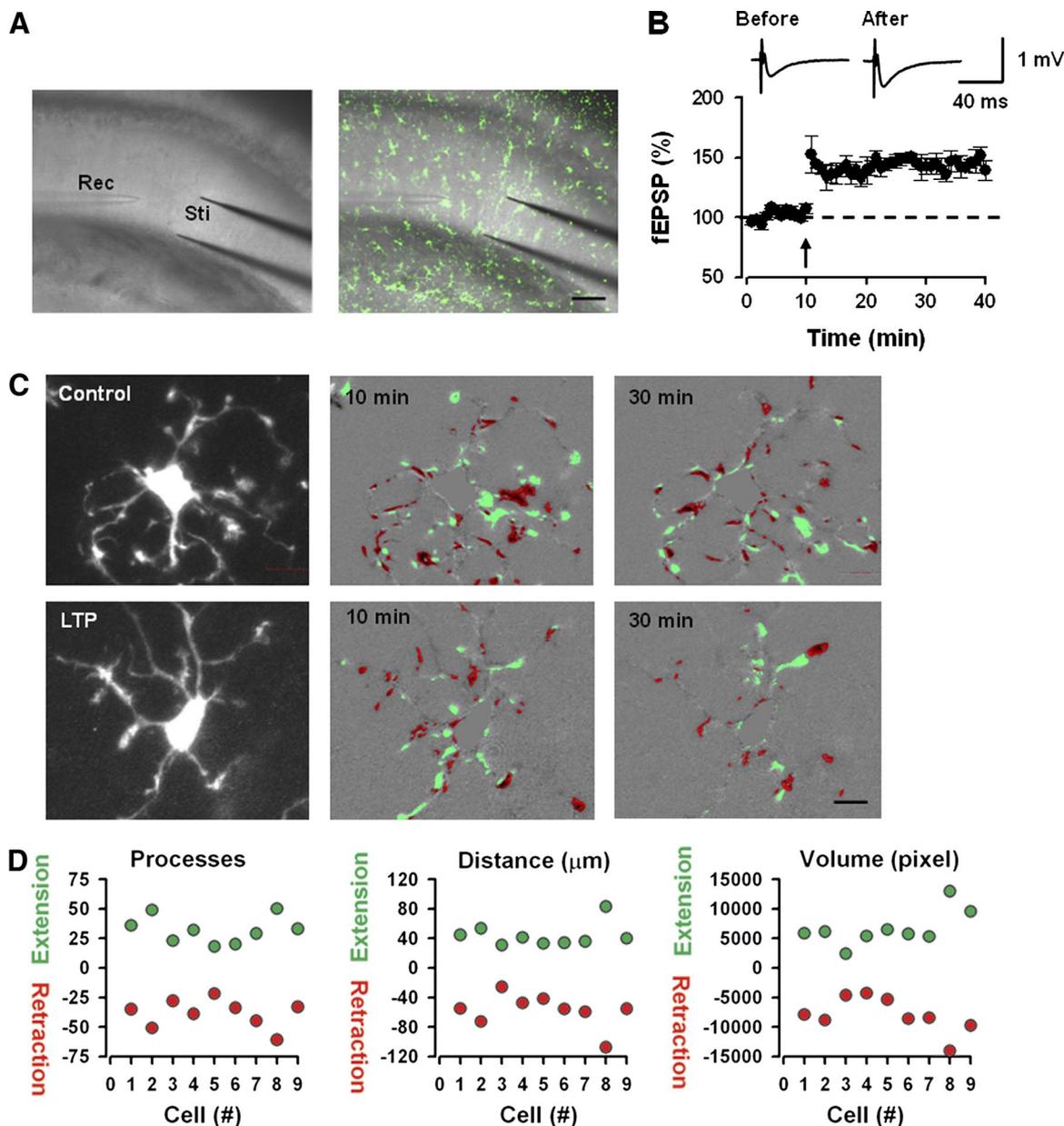

FIG. 3. Long-term potentiation (LTP) induction did not affect microglia motility in the hippocampus. A: representative images showing stimulation and field recording in the hippocampus. Both recording electrode (Rec) and stimulating electrode (Sti) were placed in stratum radiatum (*left*). Merged DIC image with GFP-labeled microglia showing the microglia is evenly distributed in the hippocampus (*right*). Scale bar, 80 μm. B: 2 trains of HFS (100 Hz, 1 s) at 20-s intervals induced LTP of field excitatory postsynaptic potentials (fEPSPs). *Insets*: averages of 6 fEPSPs at baseline response and 30 min after LTP induction (arrow). The dashed line indicates the mean basal response. C: sample images showing the extension and retraction of microglia in control (*top*) and LTP induction groups (*bottom*). Right image is showing the microglia at 0 min in each group. To obtain the extension and retraction of microglia, difference of 2 consecutive images were generated. The extended portions were marked in green, and retraced portions were marked in red. Difference at time point of 10 (*middle*) and 30 min (*right*) is shown. Scale bar, 5 μm. D: extension (green dots) and retraction (red dots) of microglia in individual microglia during the first 2 min in control group. The number, distance, and volume of extension were counted.

inward currents (12.2 ± 1.7 pA, *n* = 6) and outward currents (40.5 ± 3.3 pA, *n* = 6) in microglia in the hippocampus at a holding potential of −20 mV. However, only small outward currents were observed in CA1 pyramidal neurons (7.5 ± 1.3 pA, *n* = 6).

A time lapse confocal imaging technique was used to study the microglial responses to glutamate, GABA, or ATP (Fig. 2*B*). After local application of one of these agonists, microglia around the site of pipette were imaged every 1 min for 30 min. We found that neither glutamate (1 mM, *n* = 6) nor GABA (1 mM, *n* = 6) caused obvious microglial chemotaxis. However, immediately after local application of ATP (1 mM), microglia were responding with moving bulbous terminals of processes toward the pipette (*n* = 10; Fig. 2*B*). These results indicate that microglia show chemotactic responses to ATP but not glutamate or GABA, which are in parallel with electrophysiological data.

It is well known that hippocampal CA1 pyramidal neurons undergo activity-dependent synaptic plasticity (Bliss and Collingridge 1993; Malenka and Bear 2004). However, microglial responses associated with synaptic plasticity are unknown. To address this question, simultaneous fielding recordings and time lapse confocal imaging were used (Fig. 3*A*). Field excitatory postsynaptic potentials (fEPSPs) were recorded by stimulating in the stratum radiatum (0.02 Hz). At the same time, basal motility of microglia around recording electrode (~100 μm away) was imaged every 2 min for 40 min. After recording baseline fEPSPs and motilities for 10 min, LTP was induced by strong tetanus (100 Hz, 1s twice at 20-s interval). fEPSPs and microglia motilities were recorded for 30 min after the tetanic stimulation. Strong tetanus induced a reliable LTP in hippocampal slices (30 min after induction, 144.3 ± 6.8% of baseline response, *n* = 9; Fig. 3*B*). However, no obvious change was found in the shape of cell body or main branches of microglia before and after LTP induction (*n* = 9; Fig. 3*C*). Furthermore, there is no microglial chemotaxis toward the

stimulating electrode or CA1 layers (*n* = 9), suggesting strong tetanus did not induce chemotaxis or injury in slices.

Next, to study if strong tetanus alters microglial motilities, we analyzed the extension and retraction of microglia processes every 2 min before and after LTP induction. Processes of ramified microglia are very dynamic, showing rapid extensions and retractions with a speed of ~1 μm/min (Davalos et al. 2005; Wu et al. 2007b). Three parameters, including the number, distance, and volume of extending and retracting microglial processes, were calculated every 2 min. Nine microglia from nine slices were counted in each group. We found that the extension and retraction of microglia are variable among individual microglia. Figure 3*D* shows the extension and retraction of each microglia during the first 2 min of baseline observing time in the control group. Under control conditions without LTP induction, there is a "rundown" in microglial motilities, showing gradually declined extension and retraction during a 40-min recording (*n* = 9; Fig. 4). In the LTP induction group, similar rundown of microglial motilities was found (*n* = 9; Fig. 4). No significant difference in microglial motilities, such as the number (22.1 ± 4.3 in control, 20.6 ± 3.1 in LTP group, *P* > 0.05), distance (27.5 ± 5.5 μm in control, 29.9 ± 6.1 μm in LTP group, *P* > 0.05), or volume (72.6 ± 7.5% in control, 69.0 ± 6.2% in LTP group, *P* > 0.05) of extension were observed at the time point of 20 min between the control and LTP induction groups (Fig. 4*A*). Similarly, there was no significant difference in all these parameters of retraction between the two groups (*n* = 9 for each group, *P* > 0.05; Fig. 4*B*). At the time point of 40 min, the microglial motilities were similar between the two groups in either extension or retraction (*n* = 9 for each group, *P* > 0.05; Fig. 4). These results suggest that LTP induction did not affect short-term or long-term microglial motilities.

DISCUSSION

In this study, we performed a combination of time lapse confocal imaging and electrophysiology in resting microglia,
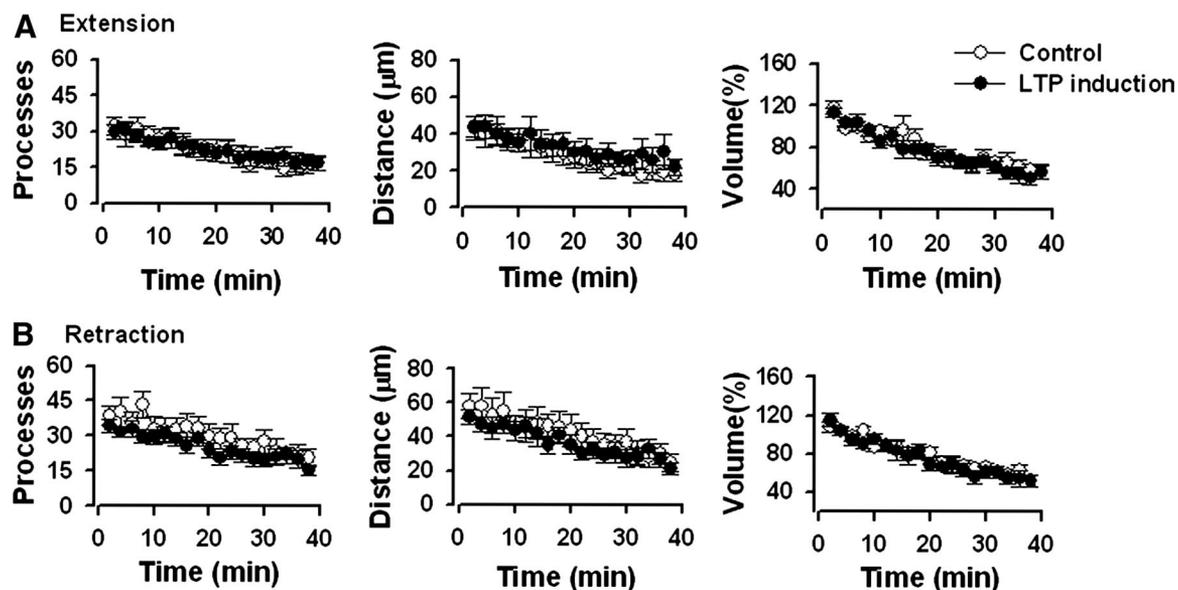

FIG. 4. LTP induction did not affect microglia motility in the hippocampus. *A*: extension of microglia in LTP induction group is similar to that in control group. The number, distance, and volume of extension were measured every 2 min for 40 min. Note that the rundown of microglial extension was found in both groups. *B*: retraction of microglia in LTP induction group is similar to that in control group.

showing that these microglial cells do not respond to neuronal activities and synaptic plasticity. Microglia could be activated by degenerating synapse or neurons and are regarded as a sensor for pathological events in the brain (Bruce-Keller 1999; Kreutzberg 1996). However, thus far, there is no study reporting the function of resting microglia. This study is a first attempt to directly address the role of microglia in physiological processes, such as neuronal transmission and activity-dependent synaptic plasticity.

By using whole cell patch-clamp recordings, we found that resting microglia did not show any spontaneous or evoked synaptic currents. Moreover, local application of classical excitatory or inhibitory neurotransmitter, glutamate or GABA, did not induce any observable current in microglial cells. Consistent with electrophysiological data, local applications of glutamate, GABA, or HFS did not induce microglial chemotaxis. It has been reported that some AMPA and kainate receptor subunits, i.e., GluR2 to GluR5, are expressed in cultured microglial cells (Hagino et al. 2004; Noda et al. 2000). However, this study showed no functional AMPA receptor in resting microglia in acute brain slices. The possible explanation for the discrepancy may be because of different preparations used (acute brain slice vs. cell culture). Taking advantage of transgenic mice with GFP-labeled microglia, we could easily identify microglia in situ, which largely represent the properties of resting microglia in both morphology and electrophysiology (Wu et al. 2007b). However, microglia in culture dish have transformed into the amoeboid cells, which may represent the activated form of microglia. Furthermore, it has been reported that activated brain microglia expressed subunits of AMPA/kainate receptor or even NMDA receptor under pathological conditions such as ischemia (Gottlieb and Matute 1997). Similarly, increased expression of $GABA_B$ receptor, which is coupled to outward potassium current in microglia, was also observed after facial nerve axotomy (Kuhn et al. 2004). Therefore despite previous studies that reported that neuronal activities might affect microglial activation (Bessis et al. 2007; Farber and Kettenmann 2005), our results suggest that resting microglia do not show electrophysiological responses or chemotaxis to acute neuronal activities. Consistent with this notion, a recent study using in vivo two-photon imaging reported that blockade of neuronal activity with TTX did not affect microglial volume sampling (Nimmerjahn et al. 2005). However, in the same study, inhibition of $GABA_A$ receptor by bicuculline increased microglial sampling, suggesting chronically increased neuronal excitability may have effects on microglial activities (Beach et al. 1995).

It has been reported that ATP could act as a neurotransmitter in the hippocampus and in some other brain regions (Burnstock 2006; Pankratov et al. 1998). In addition, we showed that exogenous ATP induced electrophysiological responses and chemotaxis of microglia in hippocampal slices. Therefore it is possible that strong tetanus during LTP induction might cause a large liberation of ATP around the stimulating electrodes (Wieraszko 1995; Zhang et al. 2007), which would affect the motilities of microglia. However, we did not find any observable current and chemotaxis of microglia during HFS, although local application of ATP induced whole cell current and the chemotaxis, suggesting that spillover of synaptically released ATP may be largely degraded by ecto-ATPase in situ (Fields and Burnstock 2006). It has been reported that microglial

processes react to the gradient rather than to the concentration of chemoattractants (Davalos et al. 2005; Nimmerjahn et al. 2005; Wu et al. 2007b). In this study, the HFS did not cause changes in microglial motility. Therefore we could conclude that microglia are not able or not sensitive enough to respond to acute neuronal activities.

Activity-dependent synaptic plasticity is associated with the release of various neuromodulators, such as ATP, brain-derived neurotrophic factor, and tissue plasminogen activator (Hughes et al. 1999; Lessmann et al. 2003; Pang and Lu 2004; Zhuo et al. 2000), which may affect microglial activities. To test this idea, we performed a combination of the field recordings and time lapse confocal imaging of microglia in the hippocampus. We found that HFS induced LTP in CA1 neurons; however, it did not affect microglial motilities. These results indicate that microglial motilities are independent of LTP in hippocampal slices. However, our results did not exclude the possibility that LTP induction may cause biochemical changes in microglia as that occurred during the pathological insults (Bruce-Keller 1999; Gonzalez-Scarano and Baltuch 1999; Kreutzberg 1996). In addition, it has been reported that microglia could be activated in cultured brain slices (Stence et al. 2001). We observed the "rundown" of microglial motilities in acute brain slices, which may reflect the gradual activation of those microglia. Therefore there are possible contaminations of activation processes in calculating microglial motilities. Activated microglia was reported to contribute to the impairment of hippocampal LTP, such as during aging (Griffin et al. 2006; Wang et al. 2004), chronic brain inflammation (Hauss-Wegrzyniak et al. 2002), or HIV infection (Xiong et al. 1999). The mechanisms involve the release of proinflammatory cytokines from activated microglia. Since there is no selective pharmacological tool in blockade of microglial motility, future experiments are needed to elucidate whether and how motility of resting microglia may be involved in LTP induction and expression under physiological conditions. For example, it would be interesting to test whether microglia are needed for the induction of LTP. In summary, these results provide new insights into the role of resting microglia in physiological functions versus pathological conditions.

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