Chinese Journal of Physiology 56(6): 309-317, 2013
DOI: 10.4077/CJP.2013.BAB129

Glial Modulation of Vibrissal Sensory Processing in Rats

Man-Li Sun, Han-Xiao Yu, Yong Han, Jun Tian, and Yan-Qin Yu

Department of Physiology, Key Laboratory of Medical Neurobiology of Ministry of Health, Zhejiang Province Key Laboratory of Neurobiology, Zhejiang University School of Medicine, Hangzhou 310058, Zhejiang, People's Republic of China

Abstract

Interactions between neurons and glial cells in the brain have important roles in brain functions such as development and plasticity of neural circuits or functions. Glial cells are much more actively involved in brain functions than previously thought. Here, we used vibrissal stimuli to induce sensory-evoked responses and multiunit spikes in the contralateral barrel cortex in a rat model. Local application of the gliotoxin DL-alpha-aminoadipate (AA) revealed that glial cells were involved in the sensory-evoked responses. The increases in the amplitude of somatosensory-evoked potential (SEP) and multiunit sensory-evoked spike rates in barrel cortex after AA injection were dramatic. Immunohistochemical staining of brain lipid binding protein (BLBP) and NeuN showed AA decreased cell number of astrocytes but not neurons in the barrel cortex. In conclusion, our results suggested an important role for astrocyte metabolism in normal synaptic activities.

Key Words: astrocyte, barrel cortex, somatosensory cortex, vibrissal stimulation, whisker

Introduction

Barrel cortex is the main part of the rat primary somatosensory cortex (SI). It has a topographic map where each whisker is represented in the "barrels" in layer IV of the cerebral cortex. The vibrissal system of rats is capable of tactile discrimination with high resolution and has served as a useful model for studying the organization of sensory processing (35). Neural transmission of vibrissal inputs begins from the peripheral nerve, transmitted to the trigeminal ganglion, and is then relayed somatotopically from the brainstem and thalamus to the barrel cortex in the SI (7, 9, 35).

Rats or mice show high sensitivity for tactile discrimination when exploring their environment (15). For example, rats can use their whiskers to distinguish texture surfaces between 1-1.06 mm gratings (5). "Whisking" speeding over these textures could generate whisker vibrations up to 100-1,000 Hz (2, 24). The activity of neurons in the barrel cortex varies rhythmically in synchrony with rhythmic whisker movement (8, 10).

The brain is now regarded as an interactive network of neurons and glial cells instead of only a circuitry of neurons. Glial cells are much more actively involved in brain information processing than we previously thought. The relevant results have come mainly from *in vitro* experiments. In intact animals, the connections between glial cells and neurons are intact and the environment is stable. Thus, in the present study, we set out to investigate the roles of astrocytes in sensory processing *in vivo*.

DL-alpha-aminoadipate (AA) is a six-carbon homologue of L-glutamate and has gliotoxic effects both *in vivo* and *in vitro* (7, 36). Astrocytic degeneration will be observed 2 h to 3 days after AA microinjection (29). In the present study, we investigated the effects of blockade by AA on the neuronal responses in barrel cortex in order to assess the role of astrocytes in neuronal activities.

Corresponding author: Dr. Yan-Qin Yu, Department of Physiology, Key Laboratory of Medical Neurobiology of Ministry of Health, Zhejiang Province Key Laboratory of Neurobiology, Zhejiang University School of Medicine, Hangzhou 310058, Zhejiang, PRC. Tel: +86 571 8820 6271, Fax: +86 571 8820 6271, E-mail: yanqinyu@zju.edu.cn
Received: June 12, 2012; Revised (Final Version): October 8, 2012; Accepted: December 4, 2012.
 ISSN : 0304-4920. http://www.cps.org.tw

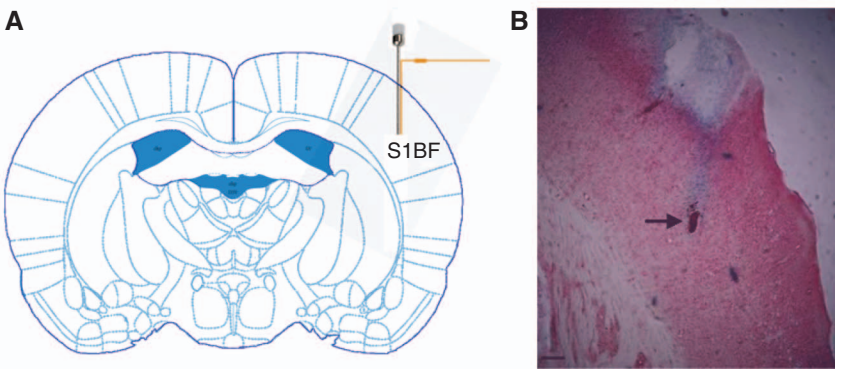

Fig. 1. Experimental setup and identification of the barrel cortex. A. A micro-syringe and an electrode are placed into the layer IV of the barrel cortex for microinjection and SEPs recording. S1BF, somatosensory cortex (barrel cortex). B. Representative example of recording sites confirmed by histological reconstruction of dye microinjection and neutral red stain. The arrow indicates the location of the electrode tip. Scale bar: 200 μm.

Materials and Methods

Animals

Male or female Sprague-Dawley rats weighing 220-300 g (Experimental Animal Center, Zhejiang University, Certificate No. 22-9601018) were raised under standard laboratory conditions of 12 h light/dark (lights on from 07:00 to 19:00), 22-26°C, and 40-70% humidity. They were provided food and water *ad libitum*. All studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals in PRC. All efforts were made to minimize suffering and to keep the numbers of animals used to a minimum.

Surgery

Rats were anesthetized with urethane (1.2 g/kg i.p. initial dose), and supplemental doses (10-15% of initial dose) were administered. Body temperature was monitored and maintained at 37°C with a controlled heating blanket and rectal thermometer (FHC, 40-90-8D, DC temperature controller). The rat was held in a stereotaxic device (Steolting, Wood Dale, IL, USA) on a floating table to minimize external vibrations. Craniotomy and durotomy were performed, and the surface of the brain was kept moist with an artificial cerebrospinal fluid. At the end of some experiments, the rats were sacrificed by urethane overdose (3 g/kg) and perfused to identify the location of microinjection.

Vibrissal Stimulation

A picospritzer (Parker Hannifin Instrumentation, Cleveland, OH, USA) controlled by a digital stimulator (PG4000A, Protech International Inc. Boerne, TX, USA) was used for whisker stimulation. The picospritzer pipette was mounted at a position allowing air puff pulse stimulation precisely vertical to the long axis of the whisker at about 10 mm from the vibrissal pad. The principal vibrissa (PV) was determined as the whisker that produced the largest amplitude and the shortest latency response.

A vibrissa was stimulated for 5 ms at 30 s intervals. Following exposure of the barrel cortex, recording probes were placed and baseline signals of both spontaneous and evoked potentials were recorded at least 30 min after surgery.

Microinjection

A small craniotomy was made according to the rat brain atlas (26). Along with the recording electrode, a syringe for microinjection (Hamilton, Reno, Nevada, USA) was inserted into layer IV of the barrel cortex at the coordinates 1.9 mm anterior to bregma, 5.5 mm lateral to the midline, and 0.8-0.9 mm below the cortical surface (26) (Fig. 1A). The microinjection volume of AA (6.4 mM) (Sigma, St. Louis, MO, USA) or vehicle was 1 μl. The injection was finished within 3 min. After some experiments, the injection site was identified by injection of 5% sky blue and neutral red stain (Fig. 1B).

Electrophysiology

Before recording, we waited at least 30 min for recovery from the surgery for all the animals. Parylene-coated tungsten microelectrodes (FHC, Bowdoin, ME, USA) with 1-2 MΩ impedance at 1 kHz were used for recording field potentials. Recordings from SI were made contralateral to vibrissal stimulation, 700-950 μm deep to the cortical surface, targeting layer IV as judged by microma-

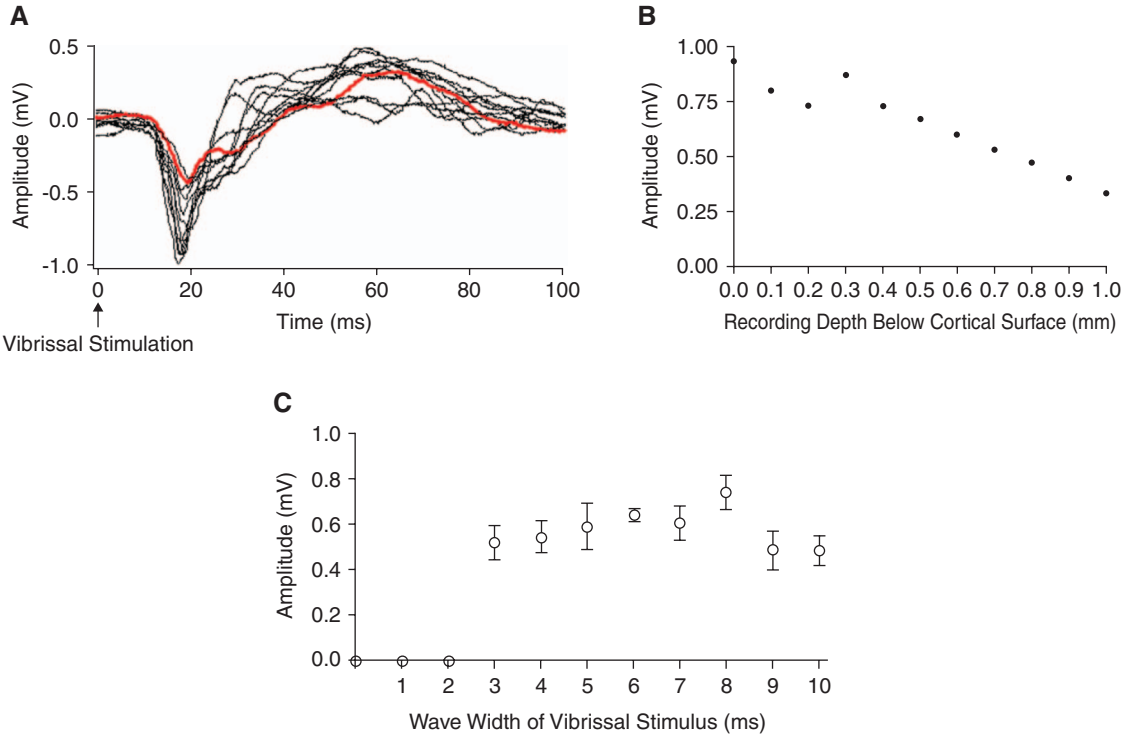

Fig. 2. Sensory responses (local field potential) evoked by a short period of vibrissal stimulation. A. Transient SEPs recorded from contralateral barrel cortex in response to vibrissal stimulation recorded in different cortical depths. Responses (normalized for amplitude) are superimposed. B. Relationship between the recording depth below cortical surface and the amplitude of the SEPs recorded. C. Relationship between SEP amplitude and the wave width of the electrical stimulus to trigger an air puff to stimulate the whisker.

nipulator readings and negative polarity of the local field potential. These depths were confirmed by histological reconstruction of electrolytic lesions in two rats. Signals were amplified 1000 × by a Model 1700 4-channel amplifier (A-M Systems Inc., Carlsborg, WA, USA) with a filter frequency range from 0.1-5 kHz for both spontaneous and evoked potentials and stored with stimulus markers. Signals were then sampled at 20 kHz with an ML795 PowerLab/4SP data acquisition system (AD Instruments, Colorado Springs, CO, USA) and stored on a hard disk in the computer for off-line analysis. The amplitude of the somatosensory evoked potentials (SEPs) was calculated from the layer IV recordings to evaluate changes in synaptic transmission. The sampling data of spontaneous field potentials were filtered by high-pass and low-pass digital filters with cut-off frequencies of 5 Hz and 400 Hz, respectively. Multiunit spike activity was filtered with cut-off frequencies of 500 Hz and 5000 Hz. Spike numbers were analyzed offline and post-stimulus histograms were generated by Neuroexplorer.

Immunohistochemical Reaction

Rats were anesthetized with 1% isoflurane and sacrificed by transcardial perfusion with ice-cold 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS). Extracted brain tissues were incubated overnight in 4% PFA/PBS and in 30% sucrose/PBS for 48 h. Sections were cut on a freezing microtome (Leica, Bensheim, Bergstraße, Germany) at 40 µm and stored in cryoprotectant at 4°C. For immunostaining, the membranes were broken by 0.3% Triton X-100 for 1 h and blocked in 10% bovine serum albumin for 1 h. Incubation with both primary and secondary antibodies was conducted at 4°C overnight. Sections were washed repeatedly in PBS after incubation with each antibody. The antibodies used were rabbit anti-brain lipid binding protein (BLBP) (1:1,000) (Chemicon, Billerica, MA, USA), rat anti-NeuN (1:200) (Chemicon), and goat anti mouse Alexa488 (1:1000), goat anti rat Cy3 (1:1000). The BLBP-immunoreactive (BLBP-IR) cells and $NeuN^+$ cells were counted in the barrel cortex of two AA-injected rats or two vehicle-injected rats. For each rat, 4 fields were selected in the barrel cortex for cell counting. Cells surrounding the injection site (about layer 4 of the barrel cortex) were counted and were compared with the cell number in the same area of the control animals, which were injected with vehicle into the layer 4 of the barrel cortex. The volume of

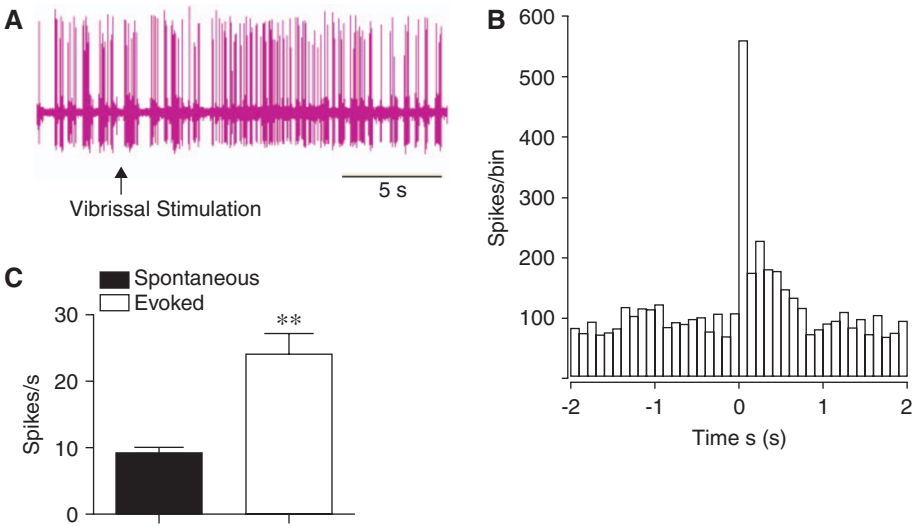

Fig. 3. Sensory responses (multiunit spikes) evoked by a short period of vibrissal stimulation. A. Representative example of multiunit spikes recorded from contralateral barrel cortex in response to vibrissal stimulation. B. Representative post-stimulus histogram of multiunit spikes recorded from contralateral barrel cortex in response to stimuli at 30-s intervals (bin width = 100 ms). C. The spontaneous and sensory evoked spike rates of the neurons in the barrel cortex.

the barrel cortex in each section was obtained by multiplying the area by the thickness of the section (40 μm) and was used to calculate the average number of $BLBP^+$ astrocytes or $NeuN^+$ neurons in the section.

Statistical Analysis

Data are expressed as means ± standard error. One way analysis of variance (ANOVA) and Student's *t*-test were used for statistical analysis. $P < 0.05$ was accepted as statistically significant.

Results

Neuronal Responses in Barrel Cortex to Vibrissal Stimulation

To explore the neural activity of the barrel cortex, a single whisker was stimulated while recording the SEPs at known depths within the cortex. Transient SEPs recorded from contralateral barrel cortex in response to vibrissal stimulation were recorded in different cortical depths. Responses, normalized for amplitude, were superimposed to show the reproducibility of waveform. The SEP waveform peaked at 90-100 ms, the onset latency of the signal was 13-15 ms, and the amplitude varied from 0.1 to 1.0 mV (Fig. 2A). In this study, we mainly recorded the field potential targeting layer IV of the barrel cortex at the coordinates 0.8-0.9 mm below the cortical surface. To have some idea about the response pattern, especially the amplitude of the SEPs in layer IV, SEPs in the barrel cortex with various depths below cortical surface were recorded. The relationship between the recording depth below the cortical surface and the amplitude of the SEPs recorded was plotted (Fig. 2B). The relationship between the SEPs and the wave widths of the electrical stimuli to trigger the air puff to stimulate the whisker was assessed (Fig. 2C). The results show that wave widths of 0-2 ms were not sufficient to trigger whisker deflection. Wave widths of 3-8 ms induced clear SEPs with a trend of increasing amplitude, while SEPs induced by wave widths of 9 and 10 ms declined. In our experiment, an electrical pulse of 5 ms was sufficient to trigger an air puff to stimulate the whisker.

To investigate the neuronal activity of a limited number of neurons in a more focused area, we recorded multiunit spikes filtered from the local field potential in response to vibrissal stimulation (Fig. 3A). An example of the post-stimulus histogram of the evoked and spontaneous multiunit response from contralateral barrel cortex in response to stimuli at 30-s intervals (bin width = 100 ms) is shown in Fig. 3B. This recording and analysis provide more information on the response pattern of the barrel cortex for the following experiment. Whisker stimuli elicited a remarkable increase of the spiking rate. The evoked spike rate (spikes/s) of neurons in barrel cortex evoked by vibrissal stimuli was 24 ± 3 and their spontaneous firing rate was 9 ± 1 ($P < 0.01$, n = 8, Fig. 3C). There was an onset response lasting about 100 ms followed by a sustained weaker increase of the spiking rate which lasted for about half a second.

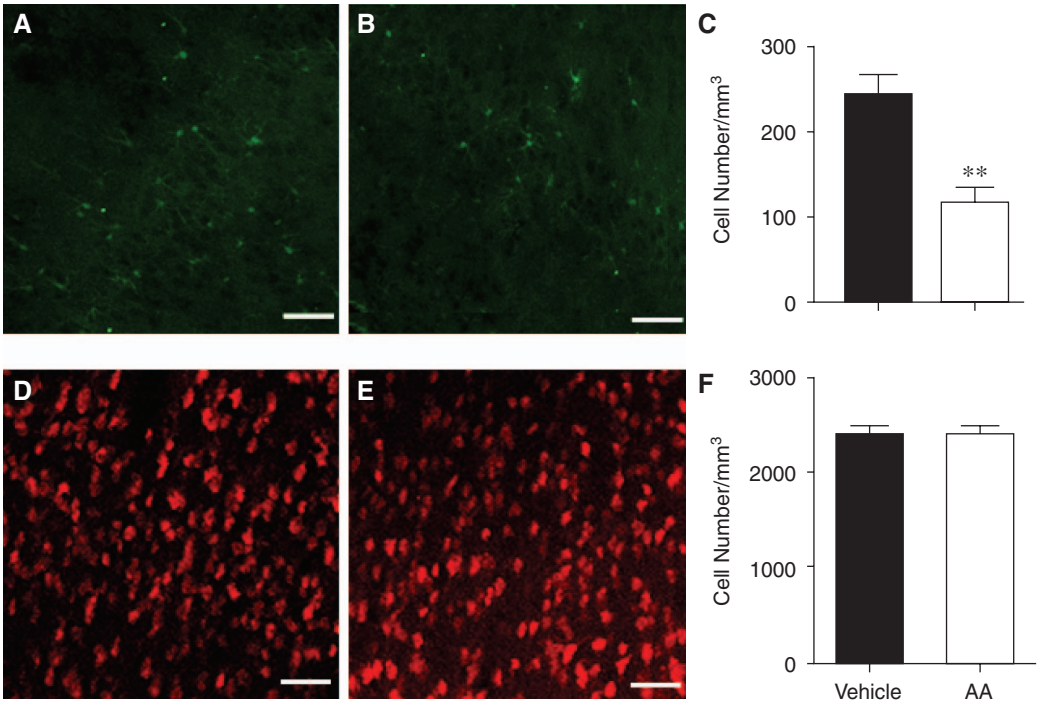

Fig. 4. BLBP-IR astrocytes and NeuN$^+$ neurons in the barrel cortex after AA injection. A-B. Representative confocal images of BLBP-IR astrocytes in the barrel cortex 2 h after injection of vehicle (A) or AA (B). Scale bars: 200 µm. C. The number of astrocytes after AA or vehicle injection. D-E. Representative confocal images of NeuN$^+$ cells in barrel cortex 2 h after injection of vehicle (D), AA (E). Scale bars: 200 µm. F. The number of NeuN$^+$ cells after AA or vehicle injection.

Decrease of Astrocytes but not Neurons in the Barrel Cortex after AA Injection

BLBP belongs to the family of fatty acid-binding proteins (FABPs). Brain FABP, also called BLBP, is widely used as a radial glia cell marker expressed in cells of the astrocyte lineage (19, 23, 31). To investigate the specific gliotoxic role of AA on astrocytes, BLBP was used to show the degeneration of astrocytes after injection of AA or vehicle. Rats were sacrificed 2 h after a 1-µl injection of AA (6.4 mM) or vehicle. In sections stained for BLBP, many BLBP-IR cells in the barrel cortex were detected by their large, green-stained nuclei (Fig. 4, A and B). In the control rats, many BLBP-IR astrocytes with small cell bodies and thin processes were found to be present in the barrel cortex (Fig. 4A). BLBP-IR astrocytes in the barrel cortex decreased significantly in the two rats injected with AA compared with vehicle. The average number of BLBP-IR astrocytes in the injection site, the barrel cortex, was $125 \pm 16/mm^3$ in AA-injected rats, while that of the control group was $261 \pm 22/mm^3$ ($P < 0.01$, n = 8 slices in 2 rats, Fig. 4C).

To exclude that microinjection of AA has any effect on neurons, we used the specific neuronal marker, NeuN, to monitor the number of neurons after injection of AA or vehicle. Rats were sacrificed 2 h after a 1-µl injection of AA at 6.4 mM, or vehicle. Many NeuN$^+$ cells in the barrel cortex were observed (Fig. 4, D and E). There were no significant changes of the number of NeuN$^+$ cells in the barrel cortex between rats injected with AA and vehicle. The average number of NeuN$^+$ cells in the injection site, the barrel cortex, was $2512 \pm 86/mm^3$ in the AA-injected rats, while that of the control group was $2553 \pm 64/mm^3$ ($P > 0.05$, n = 8 slices in 2 rats, Fig. 4F). These results show that microinjection of AA has selective toxic effects on astrocytes but not on neurons.

Increased Neuronal Responses in the Barrel Cortex after AA Microinjection

To investigate the role of glia in sensory process *in vivo*, we recorded the sensory- evoked responses in the barrel cortex after local AA injection. First, a stable baseline recording was obtained by stimulating the PV at 30-s intervals for a minimum of 30 min and recording SEPs from the contralateral cortex. Data were averaged every 3 minutes, so that the 30-min baseline was composed of 10 data points. After a stable baseline recording, AA was then injected into the barrel cortex.

Injection of AA caused an increase of the neu-

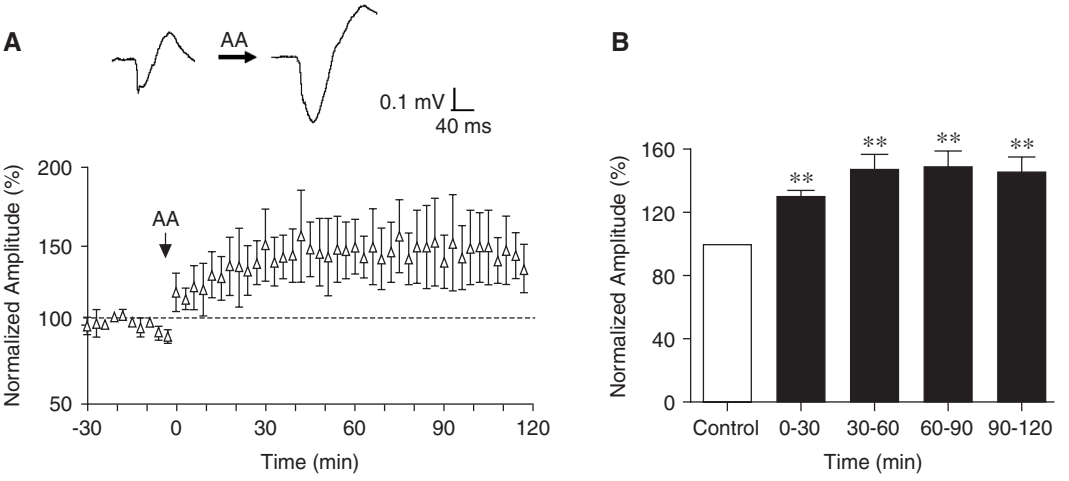

Fig. 5. Sensory-evoked responses (local field potential) after AA injection. A. Upper panel, examples of SEP waveforms recorded over the barrel cortex at baseline and 30 min after AA injection. Lower panel, time-course of normalized SEP baseline-to-peak averages before and after AA injection. B. Pooled averages of the normalized SEP amplitudes from the baseline recordings and the recordings made between 0-30, 30-60, 60-90 and 90-120 min after the injection period. Significant difference from baseline was $*P < 0.05$ or at $**P < 0.01$.

ronal response in the barrel cortex (Fig. 5A). The normalized amplitude of the SEP was 129 ± 5% within the first 30 min after AA injection ($P < 0.05$, n = 8), 146 ± 9% within 30-60 min after AA injection ($P < 0.01$, n = 8), 148 ± 11% within 60-90 min after AA injection ($P < 0.01$, n = 8), and 144 ± 10% within 90-120 min after injection ($P < 0.01$, n = 8, Fig. 5B).

AA also caused long-lasting increases in the multiunit spikes in each 30-min period. The spontaneous firing rate increased from 10 ± 2 Hz to 24 ± 9 Hz within 2 h after injection ($P < 0.01$, n = 8). The evoked firing rate increased from 10 ± 3 Hz to 20 ± 6 Hz within 2 h after injection ($P < 0.01$, n = 8). The normalized evoked spikes every 30 min gradually increased to 175 ± 27% at 0-30 min, 212 ± 54% at 30-60 min, 290 ± 68% at 60-90 min ($P < 0.05$, n = 8), and 294 ± 85% at 90-120 min after AA injection ($P < 0.05$, n = 8) (Fig. 6). The normalized spontaneous spikes every 30 min after AA injection increased to 208 ± 46% at 0-30 min, 275 ± 78% at 30-60 min, 294 ± 91% at 60-90 min, to 329 ± 114% at 90-120 min after AA injection (Fig. 6).

Injection of vehicle into the barrel cortex did not change the sensory-evoked responses. The normalized amplitude of the SEP was 100 ± 5% at baseline, 99 ± 4% within the first 30 min after vehicle injection ($P > 0.05$, n = 6) and 98 ± 4% within 2 h after injection ($P > 0.05$, n = 6).

Discussion

The sensory information from thalamocortical afferents inputs to the spiny stellate neurons in layer IV of the barrel cortex, and relays to other cortical layers within the column (22). Previous studies have shown that there are frequency-specific SI neurons in the barrel cortex (24). Rat SI neurons are sensitive to velocity of whiskering vibration (28).

Although astrocytes are the numerous cell types in the brain, evidence for their activation during physiological sensory activities is still insufficient. It is now well known that Ca^{2+} signals in astrocytes can be induced by neuronal activities and astrocytes release neuroactive substances to affect neuronal activities in turn (30, 34). The anatomical and functional organization of the barrel cortex provides a suitable model to study neuroglial interaction (13). In this study, we chose the barrel cortex as a preparation to study the role of astrocytes in either sensory input-evoked excitatory neuronal activities or spontaneous activities. AA is a homolog of glutamate and an intermediate in the metabolism of lysine which occurs naturally in the brain (6). Over two decades ago, Olney and colleagues (1971) noted the gliotoxic effects of AA in a research testing the neurotoxicity of a number of chemical compounds (7, 25, 36). After that, AA was proposed to be a selective toxin for astrocytes and related glial cells. Intrastriatal injections of AA in adult rats caused rapid degeneration in astrocytes with a loss of structural integrity at the ultrastructural level (32). It showed loss of GFAP-immunostaining area in the hippocampus 2 h after AA injection, and the loss became more obvious after 1 day. Our immunostaining results are in accordance with their result from hippocampus (29). The confirmed effectiveness and cellular specificity of AA *in vivo* reported in other studies or in the present study have made AA a potentially important experimental

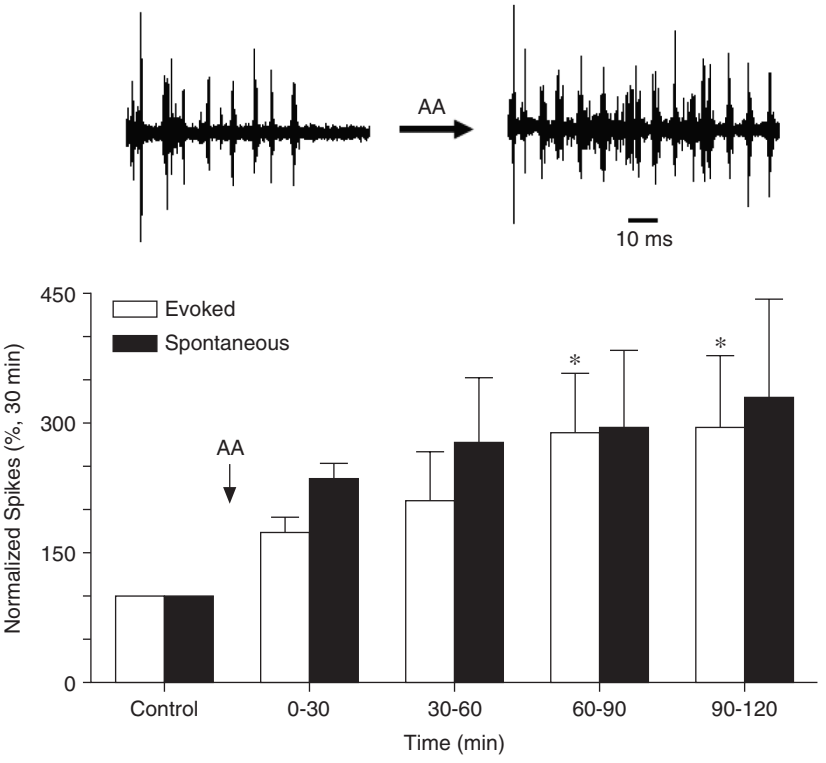

Fig. 6. Sensory-evoked responses (multiunit spikes) after AA injection. Upper panel, examples of multiunit spikes recorded over the barrel cortex at baseline and 30 min after AA injection. Lower panel, pooled averages of the normalized numbers of evoked or spontaneous multiunit spikes from the baseline recordings and the recordings made between 0-30, 30-60, 60-90 and 90-120 min after the injection period. Significant difference from baseline was $*P < 0.05$.

tool for investigating the functional significance of astrocytes (7, 36). Most studies about the role of astrocytes on neuronal network have been limited to cell cultures or *in vitro* preparations such as hippocampal slices or isolated retina (33). In physiological conditions for *in vivo* models, astrocytes were also shown to be involved in modulating neuronal excitability, activity and plasticity (15, 33). Whisker stimulation increases astrocytic cytosolic calcium (Ca^{2+}) in the barrel cortex of adult mice (34). But the role of astrocytes in sensory processing is still unclear. Our results revealed a role of astrocytes in sensory neuronal processing *in vivo*.

The general physiological importance of astrocytic signaling in modulating neuronal synaptic activity has already been unraveled (1, 11, 27). In mice, sensory stimulation elicited by 24-h whisker stimulation causes significant changes in the astrocytic sheathing of glutamate synapses (12). Astrocytes also release several neuroactive molecules, such as glutamate, D-serine, ATP, taurine and cytokine-like tumor necrosis factor-alpha to affect neurons (17, 33). Astrocytes are the primary source of ATP in the brain. ATP is important in integrating the neuron-glial networks. Astrocytes also release ATP tonically to modulate neuronal activities (20). ATP and astrocyte-released ATP and its metabolite, adenosine, altered neuronal synaptic strength (14, 37). Several recent reports support the notion that the primary action of astrocytes is to depress network activity by releasing ATP or adenosine (18, 37). In addition, astrocytes potentiate inhibitory transmission in a kainate receptor-dependent pathway (18). ATP-mediated astroglial calcium waves stimulate the release of glutamate and D-serine, which have contrary actions to ATP/adenosine, providing a different mechanism by which astrocytes could strengthen or weaken synaptic transmission (4).

Under experimental conditions, AA can influence glutamatergic neurotransmission (16). It can increase excitatory tone, which is caused in part by an elevation in synaptic glutamate concentrations (16). And the elevation of extracellular glutamate is able to increase the release of ATP, thus its metabolite-adenosine (3, 21).

There is another possibility to be involved in the effect of AA. AA can degrade astrocytes after 2 h, the cell debris may be retained *in situ* and the cytosolic content could be released to extracellular space to affect neuronal response. Furthermore, a lack of astrocytes means insufficiency in ion (mainly K^+) buffering and glutamate re-uptake capabilities. The increased

neuronal activities may be due to changes of microenvironment and the lack of astrocytic gliotransmission.

Hence, the increase of the neuronal responses after AA administration in our results strongly suggests that glial cells modulate sensory neuronal responses in the barrel system *in vivo*. Our results showed that astrocytes may function as a negative-feedback mechanism mediating excitatory transmission in the normal brain.

Acknowledgments

This work was supported by the National Natural Science Foundation of PRC (81371458, 30870834) and the National Key Technology R&D Program of the Ministry of Science and Technology of PRC (2012BAI01B08) and the Zhejiang Provincial Natural Science Foundation of PRC (Y2110057) and the Zhejiang Province Medicine and Sanitation Research Foundation (No.2008A042). We thank Professor I.C. Bruce and Dr. Xi Wang for discussions and reading the manuscript.

References

1. Agulhon, C., Fiacco, T.A. and McCarthy, K.D. Hippocampal short- and long-term plasticity are not modulated by astrocyte Ca^{2+} signaling. *Science* 327: 1250-1254, 2010.
2. Andermann, M.L., Ritt, J., Neimark, M.A. and Moore, C.I. Neural correlates of vibrissa resonance: band-pass and somatotopic representation of high-frequency stimuli. *Neuron* 42: 451-463, 2004.
3. Brückner, E., Grosche, A., Pannicke, T., Wiedemann, P., Reichenbach, A. and Bringmann, A. Mechanisms of VEGF- and glutamate-induced inhibition of osmotic swelling of murine retinal glial (Müller) cells: indications for the involvement of vesicular glutamate release and connexin-mediated ATP release. *Neurochem. Res*. 37: 268-278, 2012.
4. Butt, A.M. ATP: a ubiquitous gliotransmitter integrating neuron-glial networks. *Semin. Cell Dev. Biol*. 22: 205-213, 2011.
5. Carvell, G.E. and Simons, D.J. Task- and subject-related differences in sensorimotor behavior during active touch. *Somatosens. Mot. Res*. 12: 1-9, 1995.
6. Chang, Y.F. Lysine metabolism in the human and the monkey: demonstration of pipecolic acid formation in the brain and other organs. *Neurochem. Res*. 7: 577-588, 1982.
7. Chen, F.L., Dong, Y.L., Zhang, Z.J., Cao, D.L., Xu, J., Hui, J., Zhu, L. and Gao, Y.J. Activation of astrocytes in the anterior cingulate cortex contributes to the affective component of pain in an inflammatory pain model. *Brain Res. Bull*. 87: 60-66, 2012.
8. Crochet, S. and Petersen, C.C.H. Correlating whisker behavior with membrane potential in barrel cortex of awake mice. *Nat. Neurosci*. 9: 608-610, 2006.
9. Diamond, M.E. Somatosensory thalamus of the rat. 11, New York: Plenum Press. 1995.
10. Fee, M.S., Mitra, P.P. and Kleinfeld, D. Central versus peripheral determinants of patterned spike activity in rat vibrissa cortex during whisking. *J. Neurophysiol*. 78: 1144-1149, 1997.
11. Fiacco, T.A., Agulhon, C., Taves, S.R., Petravicz, J., Casper, K.B., Dong, X., Chen, J. and McCathy, K.D. Selective stimulation of astrocyte calcium *in situ* does not affect neuronal excitatory synaptic activity. *Neuron* 54: 611-626, 2007.
12. Genoud, C., Quairiaux, C., Steiner, P., Hirling, H., Welker, E. and Knott, G.W. Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. *PLoS Biol*. 4: e343, 2006.
13. Giaume, C., Maravall, M., Welker, E. and Bonvento, G. The barrel cortex as a model to study dynamic neuroglial interaction. *Neuroscientist* 15: 351-366, 2009.
14. Gordon, G.R.J., Iremonger, K.J., Kantevari, S., Ellis-Davies, G.C.R., MacVicar, B.A. and Bains, J.S. Astrocyte-mediated distributed plasticity at hypothalamic glutamate synapses. *Neuron* 64: 391-403, 2009.
15. Guic-Robles, E., Jenkins, W.M. and Bravo, H. Vibrissal roughness discrimination is barrelcortex-dependent. *Behav. Brain Res*. 48: 145-152, 1992.
16. Guidetti, P. and Schwarcz, R. Determination of α-aminoadipic acid in brain, peripheral tissues, and body fluids using GC/MS with negative chemical ionization. *Mol. Brain Res*. 118: 132-139, 2003.
17. Haydon, P.G. and Carmignoto, G. Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol. Rev*. 86: 1009-1031, 2006.
18. Kang, J., Jiang, L., Goldman, S.A. and Nedergaard, M. Astrocyte-mediated potentiation of inhibitory synaptic transmission. *Nat. Neurosci*. 1: 683-692, 1998.
19. Kipp, M., Gingele, S., Pott, F., Clarner, T., van der Valk, P., Denecke, B., Gan, L., Siffrin, V., Zipp, F., Dreher, W., Baumgartner, W., Pfeifenbring, S., Godbout, R., Amor, S. and Beyer, C. BLBP-expression in astrocytes during experimental demyelination and in human multiple sclerosis lesions. *Brain Behav. Immun*. 25: 1554-1568, 2011.
20. Koizumi, S., Fujishita, K., Tsuda, M., Shigemoto-Mogami, Y. and Inoue, K. Dynamic inhibition of excitatory synaptic transmission by astrocyte-derived ATP in hippocampal cultures. *Proc. Natl. Acad. Sci. USA* 100: 11023-11028, 2003.
21. Loiola, E.C. and Ventura, A.L.M. Release of ATP from avian Müller glia cells in culture. *Neurochem. Int*. 58: 414-422, 2011.
22. Lübke, J. and Feldmeyer, D. Excitatory signal flow and connectivity in a cortical column: focus on barrel cortex. *Brain Struct. Funct*. 212: 3-17, 2007.
23. Mita, R., Coles, J.E., Glubrecht, D.D., Sung, R., Sun, X. and Godbout, R. B-FABP-expressing radial glial cells: the malignant glioma cell of origin? *Neoplasia* 9: 734-744, 2007.
24. Neimark, M.A., Andermann, M.L., Hopfield, J.J. and Moore, C.I., Vibrissa resonance as a transduction mechanism for tactile encoding. *J. Neurosci*. 23: 6499-6509, 2003.
25. Olney, J.W., Ho, O.L. and Rhee, V. Cytotoxic effects of acidic and sulfur containing amino acids on the infant mouse central nervous system. *Exp. Brain Res*. 14: 61-76, 1971.
26. Paxinos, G. and Watson, C. The rat brain in stereotaxic coordinates. San-Diego: Academic Press. 1998.
27. Petravicz, J., Fiacco, T.A. and McCarthy, K.D. Loss of IP_3 receptor-dependent Ca^{2+} increases in hippocampal astrocytes does not affect baseline CA1 pyramidal neuron synaptic activity. *J. Neurosci*. 28: 4967-4973, 2008.
28. Pinto, D.J., Brumberg, J.C. and Simons, D.J. Circuit dynamics and coding strategies in rodent somatosensory cortex. *J. Neurophysiol*. 83: 1158-1166, 2000.
29. Rodríguez, M.J., Martínez, S.M., Bernal, F. and Mahy, N. Heterogeneity between hippocampal and septal astroglia as a contributing factor to differential *in vivo* AMPA excitotoxicity. *J. Neurosci. Res*. 77: 344-353, 2004.
30. Serrano, A., Haddjeri, N., Lacaille, J.C. and Robitaille, R. GABAergic network activation of glial cells underlies hippocampal heterosynaptic depression. *J. Neurosci*. 26: 5370-5382, 2006.
31. Sibbe, M., Forster, E., Basak, O., Taylor, V. and Frotscher, M., Reelin and Notch1 cooperate in the development of the dentate gyrus. *J. Neurosci*. 29: 8578-8585, 2009.
32. Takada, M. and Hattori, T. Fine structural changes in the rat brain

after local injections of gliotoxin, alpha-aminoadipic acid. *Histol. Histopathol*. 1: 271-275, 1986.

33. Volterra, A. and Meldolesi, J. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat. Rev. Neurosci*. 6: 626-640, 2005.
34. Wang, X., Lou, N., Xu, Q., Tian, G.F., Peng, W.G., Han, X., Kang, J., Takano, T. and Nedergaard, M. Astrocytic Ca^{2+} signaling evoked by sensory stimulation *in vivo*. *Nat. Neurosci*. 9: 816-823, 2006.
35. Woolsey, T.A. and Van der Loss, H. The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex: the description of a cortical field composed of discrete cytoarchitectonic units. *Brain Res*. 17: 205-242, 1970.
36. Zhang, G.H., Lv, M.M., Wang, S., Chen, L., Qian, N.S., Tang, Y., Zhang, X.D., Ren, P.C., Gao, C.J., Sun, X.D. and Xu, L.X. Spinal astrocytic activation is involved in a virally-induced rat model of neuropathic pain. *PLoS One* 6: e23059, 2011.
37. Zhang, J.M., Wang, H.K., Ye, C.Q., Ge, W., Chen, Y. and Jiang, Z.L. ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. *Neuron* 40: 971-982, 2003.