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Whisker Tactile Adaptation Is Encoded by Inactivity and Asynchrony of Network Neurons and Astrocytes in Barrel Cortex Through AMPAR Desensitization*

ZHAO Jun[1,2], WANG Jin-Hui[1,2)**]
([1] *State Key Laboratory for Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing* 100101, China;
[2] *University of Chinese Academy of Sciences, Beijing* 100049, China)

Abstract Adaptation to sensory inputs in animals influences their awareness and reactions to environment changes. The attenuated activities at all of the levels in sensory pathways, such as sensory receptors, afferent nerves and central nervous system, are likely associated with sensory adaptation. The signal encodings in cortical network neurons and astrocytes for sensory adaptation remain unclear. With the methods of two-photon cellular calcium imaging, electrophysiology and pharmacology in mice, we analyzed the dynamics of the barrel cortical neurons and astrocytes in response to repetitive inputs from whisker tactile. The paired stimuli in identical features to the mouse whiskers induced the activities of neurons and astrocytes in the barrel cortex to be spatially attenuated and temporally asynchronized. The interaction between these neurons and astrocytes became less coordinated. The downregulation in the spatial and temporal activities of the neurons and astrocytes was significantly reversed by locally using an inhibitor of glutamatergic AMPA-receptor(AMPAR) desensitization. Therefore, the inactivity and asynchrony of network neurons and astrocytes in barrel cortex through AMPAR desensitization are associated with encoding the adaptation of whisker tactile sensation.

Key words neuronal network, astrocyte, barrel cortex, whisker, adaptation, AMPA-receptor, *in vivo* two-photon imaging
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Sensory adaptation is a physiological phenomenon in the animals[1]. This short-term sensory plasticity affects their behaviors and cognitions to environmental changes[2]. In terms of its mechanism, much attention was paid to studying sensory receptors and afferent nerves[1]. To the role of cerebral cortex in sensory adaptation, the investigations in computational simulation and experiments indicated neuronal inactivity and synaptic depression during the sensory adaptation [3]. How a population of nerve cells in sensory cortex coordinately work together to encode sensory adaptation remains unclear.

The recording of electrical signals by multiple electrodes was used to study cellular mechanism underlying the sensory adaptation in visual cortex [4], auditory cortex [5] and barrel cortex [3]. Brief visual adaptation was featured as the reduced mean and variation in the correlation of network neurons [4]. Whisker tactile adaptation was associated with the attenuation of neuronal responses and correlation[3]. On the other hand, repetitive stimuli to whiskers led to more adaptation in inhibitory synapse to shift neural balance toward the excitation [6]. These inconsistent results implied that neuronal encodings for the adaptation of these sensations might not follow a simple rule. Moreover, these studies did not reveal how the neurons and astrocytes in sensory cortex

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**Corresponding author.
Tel: 86-10-64888472, E-mail: jhw@sun5.ibp.ac.cn
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interact each other to encode sensory adaption

A current study by two-photon cellular Ca^{2+} imaging showed that some neurons and astrocytes in barrel cortical networks to a given sensory input were upregulated, but others were downregulated in their activity patterns and functional connections. An increase of input frequency changed the weight of activity patterns in these nerve cells[7]. We hypothesized that the encoding of the sensory adaptation was fulfilled by reorganizing their activity patterns and functional connections. By using the approaches of two-photon cellular calcium imaging, electrophysiology and pharmacology *in vivo*, we analyzed the temporal and spatial patterns of barrel cortical neurons and astrocytes in response to whisker tactile stimuli with identical features that induced sensory adaptation, and studied potential mechanisms underlying the change of activity patterns during sensory adaption.

1 Materials and methods

1.1 The procedures for examining the adaptation of whisker tactile inputs in mice

The entire procedures were approved by the Institutional Committee of Animal Care Unit in the Administration Office of Laboratory Animals at Beijing China (B10831). FVB mice (25～35 postnatal day, 25～35PND) were placed in a customer-made cage with the partial fixation of their heads (Figure 1) and the restriction of their body movements. The major whiskers of mice were stimulated by puffing air-flow pulses toward whiskers in a caudal-to-rostra direction with a consistent intensity and frequency, *i.e.*, the protocol of stimulating whiskers was to make their deflection. The deflection in a caudal-to-rostra direction is more similar to the natural movement of whiskers. The responses of their whiskers to stimuli were monitored by a high-speed CCD video camera (100 frames/s). All of the experiments were conducted in a quiet room with a temperature at 22℃. The cares were taken including no stress and circadian disturbances to mice.

Major whiskers on the one side were stimulated by paired burst-pulses, but those on other side were used as the control. Whisker deflections were done by using sequential air-puffing pulses (50 psi, 50 ms) through a tiny steel tube that was mounted on a micromanipulator and controlled with costumer-made LabVIEW program. The stimulus patterns were the paired burst-pulses in consistent intensity and frequency. The frequency patterns in these paired-bursts were 10 and 10 Hz (10-to-10 Hz), closely to the natural frequency of whisker fluctuated motion[8]. The use of this frequency is easy for mice to show the adaptation of whisker sensation since the adaptation of sensory nerves occurs under natural conditions. Burst-pulse intervals were 10 seconds and each of bursts lasted for 10 seconds. To avoid the stimuli to the skin/furs, a tip of stimulator was positioned in a way that it did not blow on the mouse snouts.

The response strength of whiskers was quantified based on their retraction duration after stimuli. In analyzing the images of whisker retraction, we defined the positions of major whiskers as resting and retraction, based on the averaged position angles that were the differences of whisker positions between the resting and retraction. A zero degree indicates the end of whisker retraction, *i.e.*, a resting state. The longer duration denotes the stronger response, or vice versa. In other words, the reduction of retraction duration was thought as sensory adaptation.

1.2 Animal surgery and fluorescence labeling

FVB mice (25～35 PND) were anesthetized by intraperitoneal injections of urethane (1.5 g/kg). Anesthetic depth was judged based on lack of reflexes in pinch withdrawal and blink eyelid, and was maintained by giving the supplemental dosages of urethane (0.25 g/kg) throughout experiments. Body temperature was maintained by using a computer-controlled heating blanket at 37℃. The location of barrel cortex was identified by a distributional map of superficial vessels and confirmed by histological reconstruction after each experiment [7, 9–10]. It is noteworthy that the dura was intact in all experiments, and the care was taken to avoid any damage to superficial vessels and cortices.

Oregon Green BAPTA-1-AM (OBG-1, Invitrogen, USA), Ca^{2+} indicator, was used to measure the activities of both neurons and astrocytes. OGB-1 was dissolved in DMSO and 20% Pluronic F-127 (2 g Pluronic F-127 in 10 ml DMSO, Invitrogen) to have its stock solution at 10 mmol/L, which was diluted in ACSF to yield its final concentration at 1 mmol/L. OBG-1 solution was injected into layers Ⅰ～Ⅱ of barrel cortex by a pressure (100 kPa, 5 min) through a patch pipette (200 μm below the pia) to label a population of nerve cells [7]. In the meantime, 100 μmol/L sulforhodanmine-101 (SR101, Invitrogen, USA) was co-injected to label the astrocytes[11]. After

the micro-injections, this craniotomy well was filled by low-melted agarose (1%～2%) dissolved in saline and then was sealed by glass coverslip. The exposed skull was adhered to customer-made metal recording chamber by dental acrylic cement and surperfused by saline (mmol/L): 125 NaCl, 2.5 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 20 glucose (pH 7.4). The saline was warmed up to 37℃ and bubbled with 95% O_2 /5% CO_2.

1.3 Two-photon cellular imaging

Cellular Ca^{2+} imaging experiments were done 1 h after dye injections by using a two-photon confocal laser scanning microscope (Olympus FV-1000, Japan) equipped with a two-photon laser-beam generator (Mai Tai, Physical Spectrum, USA) and a scanning system. They were mounted to an upright microscope (Olympus BX61WI) with the water immersion objectives(IR-LUMPLan Fl, 40×, 0.8NA). Two-photon laser beam (810 nm) was given for an excitation of OGB-1 and SR101. The average power in laser beam for exciting fluorescents, the emission wavelengths for scanning samples and the frame rate for imaging (10 Hz, 256 pixels × 256 pixels) were locked for the measurements before and after different stimuli as well as pharmacological reagent use throughout all experiments in order to have a consistent condition in the comparisons of the results[7].

1.4 Local field potential (LFP) recording

Local field potentials were recorded in layers Ⅱ/Ⅲ of barrel cortex by using a glass pipette that contained the standard pipette solution (150 mmol/L NaCl, 3.5 mmol/L KCl, 10 mmol/L HEPES). The resistance of recording pipettes was 5～7 MΩ. The electrical signals were inputted to an AxoClamp-2B amplifier and pClamp 10 system (Axon Instrument Inc. CA USA), in which Clampex was applied for data acquisition and Clampfit for data analysis. The electrical signals were digitized at 10 kHz and filtered by low-pass at 0.1 kHz. The LFP recording and two-photon cellular imaging were performed in the identical area of barrel cortex [7], which allowed us to study the processes of neuronal encoding in barrel cortex by cellular imaging and electrophysiology.

1.5 Whisker stimuli and barrel cells′ responses

To mimic the natural whisker behavior, we used the multi-whisker stimulation protocol to induce the cortex responses [12]. All major whiskers in the contralateral sides of the imaged barrel cortices were deflected in a caudal-to-rostral direction by air-puffing during the experiments (see above) at 10 Hz. This multi-whisker stimulation with 10 Hz induced a robust neural spiking activity in a large area of barrel cortex[12]. Stimulus patterns were the paired burst-pulses, in which each of burst pulses had 10 Hz in frequency (10-to-10 Hz) and consistent intensity. Burst-pulse intervals were 10 seconds and each of bursts lasted for 10 seconds. To avoid the stimuli to skin and furs, a tip of the stimulator was positioned in a way that it did not blow on the snout. Thus, these stimulation protocols facilitated us to research network resposes in somatosensory adapation.

The amplitudes of Ca^{2+} signals were correlated positively with spike frequency, and Ca^{2+} levels in a neuron indicate its functional activity, such that spiking activity at the neurons was estimated from their somatic Ca^{2+} signals *in vivo*[13]. Different from the spikes as a functional index of neuronal activity, astrocytic activities are only associated with the changes of Ca^{2+} signals[14]. In addition, the synchrony of Ca^{2+} signals provide a measurement for the timing activities among nerve cells[15–16]. Therefore, we studied the activity patterns of barrel cortical neurons and astrocytes in response to whisker stimuli by using *in vivo* two-photon cellular imaging in order to reveal the processes of neuronal encoding in brain network, in which the peak amplitudes and temporal synchrony of Ca^{2+} signals were analyzed.

1.6 The local application of pharmacological agents *in vivo*

In order to use cyclothiazide locally, we placed a soft plastic tube (0.5 mm) onto the surface of barrel cortex. A hole was drilled on the skull about 2 mm away from the wells of two-photon imaging. The tip of this plastic tube was penetrated into the dura in a direction toward the barrel cortex without its injury. The tip of this plastic tube was seen around the edge of the wells for imaging objective lens. This plastic tube was then adhered onto the skull with dental acrylic cement. Another end of this plastic tube was connected a syringe for the micro-injections of 10 μmol/L cyclothiazide. The injected volume was controlled within 2 μl. The experiments of behavioral tasks and two-photon cellular imaging were done before and after cyclothiazide injections.

1.7 Data analyses

Ca^{2+} fluorescence signals from cellular responses to whisker stimulation were acquired by using Fluoviewer-10 software (Olympus Inc. Japan) and

analyzed in the regions of interest (ROI) from the cell bodies by using NIH ImageJ and MATLAB (MathWorks). To reduce the photon and PMT noise, a median filter (radius, 1 pixel) was used to all images. Ca^{2+} signals in cellular responses were normalized and presented as relative fluorescence changes ($\Delta F/F$). Baseline fluorescence (F) was an averaged value in the ROI before stimuli, and ΔF values were differences between Ca^{2+} signals from the evoked responses and baseline fluorescence. It is noteworthy that all of the fluorescence signals were subtracted from the noise signals of unstained blood vessels. The normalized Ca^{2+} signals were smoothed by low-pass Butterworth filter to remove low-amplitude fluctuations and to minimize distortions from fast Ca^{2+} transients [13]. The effective signals from each of active cells were judged according to a criterion that their relative fluorescence changes were greater than 2.5 SD of baseline values lasting for 500 ms. Whisker stimuli induced the robust changes of Ca^{2+} signals in barrel cortical cells. The criteria above were found effective for sorting evoked signals from noise[7].

In our study, the paired burst-stimuli were given to induce two sequential responses of calcium signals. The measuremnts of their values were standardized to be the followings. The amplitudes of response one were the difference between the baseline and peak one of calcium signals. The amplitudes of response two were the difference between the residual level of response one and peak two of calcium signals. Because the fluctuation of fluorescence signals affected the precise measurement of response amplitude, we thought the magnitude differences of two responses if their differences were above 2 SD of baseline values; however, we defined no difference in the magnitude of two responses if their net changes were less than 2 SD. If cellular response one (R1) was larger than response two (R2) above 2 SD of baseline, the pattern was defined as a decrement (R1>R2). On the other hand, R2>R1 above 2 SD was an increment. No difference in R1 and R2 was called as parallel[7]. This classification is similar to synaptic transmission patterns [17]. It is noteworthy that R2 values were the absolute changes of responses induced by stimulus two. That is, if calcium signals in response to stimulus one were not back to their baseline levels, R2 was measured as a difference between the magnitude of response two and the residual level of response one.

The pairswise cross-correlations of the normalized and smoothed Ca^{2+} signals ($\Delta F/F$) in the neurons and/or astrocytes between their pairs were analyzed as the Pearson correlation[15]. Considering two signals $x(t)$ and $y(t)$ of a real variable t, the cross-correlation r at delay d is defined as:

$$r = \frac{\sum_t [(x(t)-mx)\times(y(t-d)-my)]}{\sqrt{\sum_t (x(t)-mx)^2}\times\sqrt{\sum_t (y(t-d)-my)^2}}$$

mx and my are the means of the corresponding series. The correlation coefficients normalized to the autocorrelation at zero lag were calculated. Based on the calculations, the correlation matrices were plotted using MATLAB 7.0 [7]. As the cross-correlations between neurons from raw fluorescence traces were higher than the deconvolved traces over two folds [15], we computed the raw traces without temporal deconvolution in the neurons, similar to those in the astrocytes that have no spikes firing[18].

To study the functional connectivity among nerve cells, we converted correlation coefficient matrix (r) into binary adjacency matrix (A) by setting a threshold ($thresh$) [19]. This threshold was the mean values of correlation coefficients plus the values of two-time standard deviations corresponding to the spontaneous activities of network cells without whisker stimuli[7].

The definition of functional connection, whose threshold was set at a mean +2SD of correlation coefficients during the spontaneous activities of network cells, was based on an assumption that their activities were random (no coordination). In other words, there was no interaction or functional connection among the network cells without input signals[7].

Based on these criteria of binary adjacency matrices and the spatial positions of network cells, we plotted the graphs which were composed of a set of nodes (the cells activated by stimulus bursts) and their functional connections (lines) under the conditions of response one and two corresponding to 10 Hz stimulus burst and 10 Hz one, respectively. In these graphs of neural networks, two parameters for each cell were meaured to indicate how each cell was connected with others. Cells that connected with one at least was called as a functionally connected cell in network. The percentages of functionally connceted cells in neural network presented how many cells were functionally connected with others. If a cell connected with others, the percentages of functional connections for its

actually connected cells in the total cells were calculated to present the connection strength for each of network cells. The folmula are given below.

The method we here defined functional connection is not based on the effective synaptic responses but the statistical relationship of cellular activities. For a neuronal network consisting of activated neurons (N) in the complete graphs, the number of connctions for each neuron with others is $N-1$. The number of functionally connected neurons is n, and the averaged number of connections for each neuron with others is k. Thus, $P_n=n/N$ stands for the percentages of functionally connected neurons. $P_k=k/(N-1)$ presents the percentages of functional connections of each neuron. The astrocytes are connected tightly and widely through gap junctions, the number of connected neuron-astrocyte pairwise shows no variation during different stimulations in our experiments. In a network comprising of N neurons and M astrocytes, we calculate the percentages of the neuron-astrocytic functional connections (l) in total potential links, which is $P_l=l/(N\cdot M)$.

All data are presented as mean ±SE. Student's t tests (two-tailed, paired, or unpaired assuming unequal variances) were done in R software package, version 2.10.1(http://www.r-project.org/) to evaluate statistical significance. $P \leqslant 0.05$ is defined as statistical difference.

2 Results

Our strategies to elucidate cellular and molecular mechanism underlying the sensory adaptation are the following: The mouse model of sensory adaptation was established by seeing the weakened responses of their whiskers to the paired burst-stimuli with identical features. To investigate the role of neurons and astrocytes in encoding sensory adaptation in the mice, we analyzed the spatial and temporal responses of barrel cortical neurons and astrocytes to the paired burst-stimuli onto their whiskers by cellular Ca^{2+} imaging and electrophysiology *in vivo*. In terms of molecular mechanisms underlying neural encodings for sensory adaptation, we analyzed the activity patterns of barrel cortical neurons and astrocytes before and after local application of pharmacological reagents to barrel cortex.

The activity patterns of nerve cells analyzed from two-photon calcium imaging *in vivo* included their response strength and synchrony. Calcium signal strength was proportional to cellular activity [13]. Activity synchrony among these nerve cells was analyzed by pair-wise cross-correlations[15]. The signals from the astrocytes were isolated by labeling them with SR-101[11]. In addition, local field potentials were recorded in barrel cortex to have our data supported by cellular electrophysiology and functional imaging. The paired-burst stimulations to study sensory adaptation in whisker tactile input were composed of two sequential bursts to whiskers in 10-to-10 Hz and consistent intensity, in which each of bursts lasted for 10 seconds and inter-burst intervals were 10 seconds. It is noteworthy that 10 Hz whiskers movement is a typic behavior in rodent tactile discrimination. Repitively stimuli the multi-whiskers at 10 Hz induced cortical rapid adaptation with the depression of field potential and spikes[12]. So, we gave the long term stimuliation at 10 Hz to mimick the rodents' discrimination behavior and to exam the process on how they adapted to discriminate the identical feature they recognized before. The interval of 10 seconds was optimized based on the long decay time of evoked calcium signals, especially in astrocytes. During a 10 s interval, most calcium signals came back to the baseline. In addition, we induced tactile sensory adaptation with a longer interval up to 40 s and other frequencies of whisker stimuli as well.

2.1 The adaptation of whisker responses to sensory stimuli

In the models of sensory adaptation, we studied the responses of mouse whiskers to tactile input. The input stimuli were the paired burst-pulses of air-puffing to whiskers in identical features (*e.g.*, 10-to-10 Hz) in a caudal-to-rostra direction. Without a communication *via* "language", the intensity and duration of sensation are assessed based on behavioral reactions. For instance, whisking frequency and retraction are used to assess whisker tactile sensation[20]. Moreover, whisker retraction can be directly influenced by the barrel cortex [21], indicating that whisker tactile sensation and activity are closely associated each other. These support our usage of whisker retraction duration to evaluate tactile sensation. The responses of the whiskers were quantified based on their retraction durations after stimuli, which were recorded by a digital video camera. Once the stimuli were given, mouse whiskers retracted, such that a positional angle was formed between resting position and retraction. A zero of this

angel, *i.e.*, the whiskers were back to the resting position, indicated the end of whisker retraction. The time from a retraction to its end was counted as retraction duration. The longer duration of whisker retraction represents the stronger sensory responses, or vice versa. The shorter retraction duration in the second response versus the first one indicates the sensory adaptation.

Figure 1 illustrates whisker responses and their persistent duration after paired-burst stimuli. The images in Figure 1a show whiskers' positions under the conditions of resting as well as 1.3, 2.3 and 3.3 s after their retraction evoked by paired-pulses. Top panels demonstrate response one (R1) and bottom panels show response two (R2). The whiskers appear to retract for the longer period in R1. Statistical analyses in Figure 1b demonstrate that the retraction durations for R1 and R2 are (2.92±0.32) s and (1.62±0.25) s, respectively (asterisks, $P < 0.01$; $n=9$). The shortened retraction duration in response two versus response one indicates the adaptation of whiskers in response to sequential tactile inputs.

We then studied cellular and molecular mechanisms underlying this sensory adaptation by the multiple approaches of electrophysiology, cellular imaging and pharmacology. As barrel cortex directly controlled the retraction of the whiskers without a necessarily need of motor cortex[21], these experiments were done in the barrel cortex.

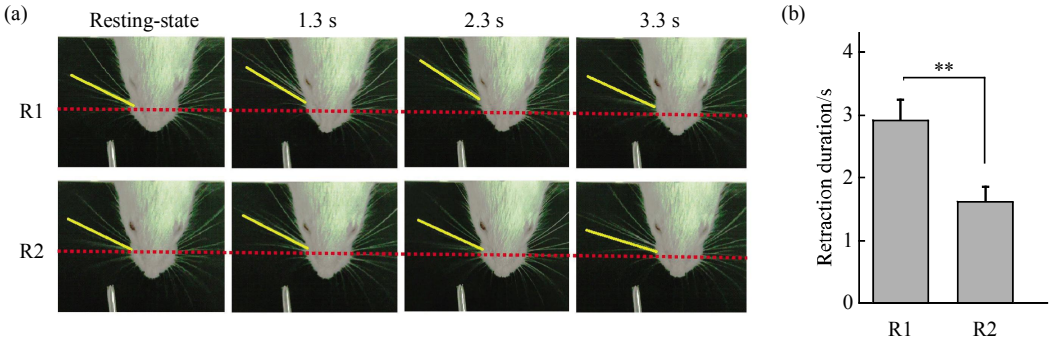

Fig. 1 The identical stimuli leads to the adaptive responses of mouse whiskers to tactile inputs

(a) The responses of whiskers to the paired burst-stimuli in identical feature. Top panels are reponse one and bottoms are reponses two. Panels from left to right are the responses under the resting and 1.3, 2.3 and 3.3 s after the stimuli. Yellow bars indicate the position of major whiskers under these conditions. (b) The statistical comparison of whisker retraction durations in reponse one and two (**$P < 0.01$).

2.2 A population of neurons is involved in encoding sensory adaptation in a decrement pattern

The activities of population neurons were monitored by using glass pipettes to record local field potentials in layers Ⅱ/Ⅲ of barrel cortex. Local field potentials and two-photon cellular imaging were recorded in the identical regions of barrel cortices[7], which allowed to obtain the convincing data about the processes of neural encodings.

Figure 2 shows the responses of barrel cortical neurons to paired burst-stimuli. The waveforms in 2a (a single trace) and 2b (a trace averaged from 10 traces) show the decreased field potentials in response two. Statistical analyses in Figure 2c show that the amplitudes of R1 and R2 are (0.21±0.02) mV and (0.16±0.01) mV, respectively (asterisks, $P<0.01$; $n=8$). The lower amplitudes in response two *vs.* response one, *i.e.*, a decrement pattern, indicates the adaptive activities of barrel cortical neurons in response to sequential tactile inputs. The result also indicates that a population of neurons is involved in encoding sensory adaptation.

Neural networks consist of neurons and glia cells, and the astrocytes play important roles in the function of neurons [7]. We subsequently investigated how neurons and astrocytes in barrel cortex coordinately encoded the sensory information for the whisker tactile adaptation.

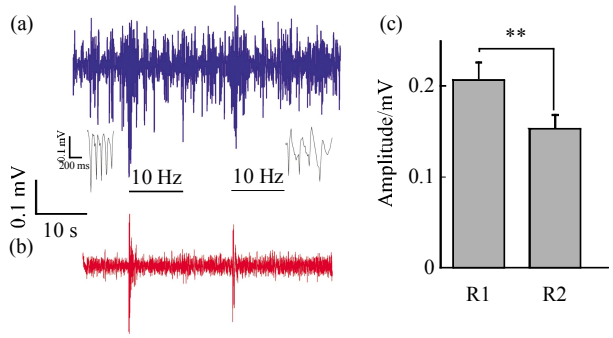

Fig. 2　The responses of barrel cortical nerve cells to the paired identical stimuli appear decrement

(a) A single trace of local field potentials in response one and two. Black traces are the two zoomed-in evoked LFPs. (b) A trace of local field potentials averaged from 10 traces. Calibration bars are 0.1 mV and 10 s. (c) The statistical data illustrate the amplitudes of response one and two at (0.21±0.035) mV and (0.16±0.02) mV (**P < 0.01; n=8).

2.3　Neurons and astrocytes encode sensory adaptation mainly through decrement pattern

The activities of individual neurons and astrocytes in barrel cortex were detected by two-photon cellular imaging *in vivo*. Their responses to the paired burst-stimuli in identical frequency and intensity were analyzed. The analyses were based on an assumption that their activity strength and synchrony are proportional to input frequency[7].

Figure 3a illustrates 3 patterns of the activity strengths of barrel cortical neurons and astrocytes in response to the paired whisker stimuli. Ca^{2+} signals from the neurons and astrocytes in response to stimulus one and two show that the neurons are activated mainly in a pattern of R1 larger than R2, *i.e.*, decrement (Figure 3b and c). The data from all of the neurons in six experiments show a high portion of the neurons to express decrement *vs*. other patterns (increment and parallel). The percentages of decrement, increment and parallel patterns are (48.13 ±7.15)% (blue bar in 3b), (16.92 ±3.03)% (red) and (34.95 ± 5.53)%, resepctively (green; P<0.001). It is noteworthy that R2–R1 is inversely and linearly correlated to R1 (r^2=0.7; Figure 3d), *i.e.*, the decrement pattern depends on response one[7, 17].

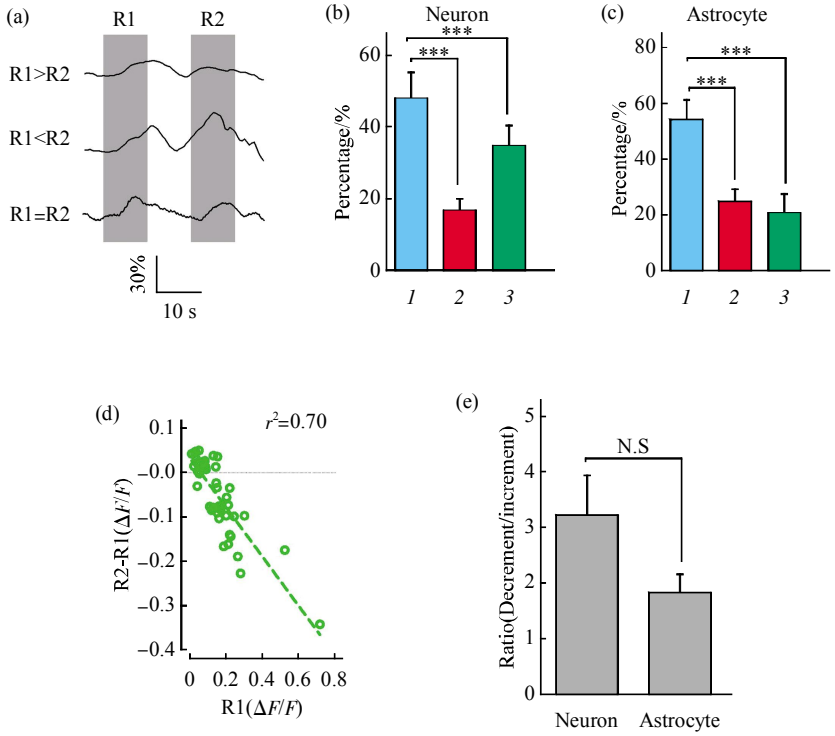

Fig. 3　Barrel cortical neurons and astrocytes show a dominant decrement in responses to the paired whisker stimuli with identical features

(a) 3 patterns of two sequential Ca^{2+} signals (R1 *vs* R2) are induced by stimilus one and two. (b) The percentages of decrement (blue), increment (red) and parallel (green) among the neurons (n=5) in response to identical paired burst-stimuli. ***P < 0.001 in student's t-test. (c) The percentages of decrement (blue), increment (red) and parallel (green) among the astrocytes (n=5) in response to identical paired burst-stimuli. ***P < 0.001. *1*: R1>R2; *2*: R1<R2; *3*: R1=R2. (d) The inverse and linear correlation between R2–R1 and R1, indicating the depedence of response patterns on response one. (e) The ratio of decrement to increment in neurons and astrocytes (P=0.2).

In terms of astrocytic responses to the paired whisker stimuli, most of the astrocytes express a decrement pattern. The percentages in decrement, increment and parallel averaged in all of the experiments (n =7) are (54.11 ±7.16)% (blue bar in Figure 3c), (24.92±4.41)% (red) and (20.97±6.65)%, respectively (green; $P < 0.01$). Similar to the neurons, barrel cortical astrocytes in response to the paired whisker stimuli in identical features mainly express decrement pattern. This indication is strengthened by Figure 3e that a ratio of the decrement to the increment is not statistically different in the neurons and astrocytes. The decrement pattern induced by the identical stimuli was comfirmed at other frequency such as 12 Hz or 8 Hz[7] additional files.

In summary, the study by recording LFP indicates the involvement of population nerve cells in encoding sensory adaptation. The data from two-photon cellular imaging show that individual neurons and astrocytes in barrel cortex are active to encode neural signals for sensory adaptation. Both studies suggest that the decrement activity pattern of nerve cells is a mechanism underlying sensory adaptation. How these neurons and astrocytes in barrel cortical networks were coordinately activated (*i.e.*, activity synchrony) to encode the sensory adaptation was further examined.

2.4 Neurons and astrocytes coordinately encode sensory adaptation through less activity synchrony

The coordinate activities of the neurons and/or astrocytes in the barrel cortex were evaluated by analyzing correlation coefficients among the pairs of neurons and/or astrocytes, in which their activities were based on the data from two-photon cellular imaging above. Although there was a limitation of low temporal resolution of calcium imaging, the correlation between cellular calcium signals was suitable to quantitatively analyze the similarity of activity [15]. The neurons in Layer Ⅱ / Ⅲ are wired together and weather the cells were drived by a same inputs or connected directly [15]. Higer similarity indicates higher synchrony of the neural activities.

Figure 4a illustrates the cross-correlations for the pairs of neurons in response to stimulus one and two. Each pixel in matrices represents peak cross-correlation for a pair of neurons, and dark-red pixels indicate the best cross-correlation (synchrony), or vice versa. The correlation coefficients in central peaks averaged from seven experiments for stimulus one and

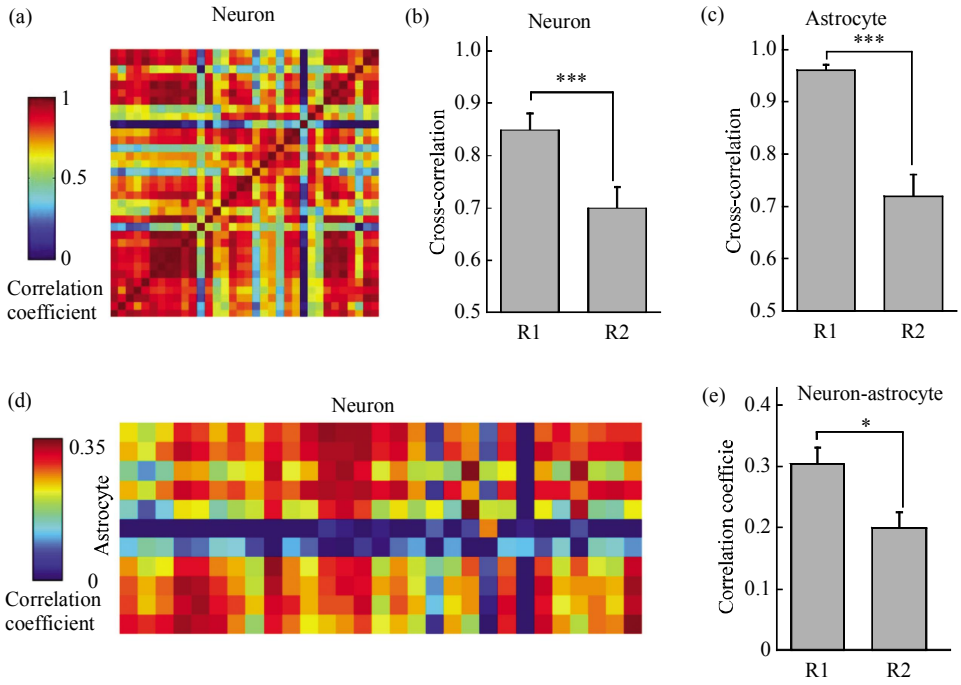

Fig. 4 The activity asynchrony of barrel cortical neurons and astrocytes is correspondent to identical paired burst-stimuli

(a) Correlation matrices show cross-correlations for each of neuron-pairs. (b) A comparison of averaged correlation coefficients for all active neuron-pairs in responses (R1 and R2) to stimulus one and two (***$P < 0.001$). (c) A comparison of correlation coefficients for all active astrocyte-pairs in responses (R1 and R2) to stimulus one and two (***$P < 0.001$). (d) The correlation matrices show cross-correlations for each of neuron-astrocyte pairs in response to stimulus one. (e) A comparison of correlation coefficients for all active neuron-astrocyte pairs in responses (R1 and R2) to identical paired burst-stimuli. *$P < 0.05$ in student's t-test.

two decrease from (0.86±0.03) to (0.68±0.03) in the neurons ($P < 0.01$, Figure 4b) and from (0.95±0.01) to (0.72±0.03) in the astrocytes ($P < 0.01$, Figure 4c). Thus, less synchronous activities among network neurons or astrocytes in response to identical stimuli may be a typical encoding process for sensory adaptation.

In addition, the neurons and astrocytes in barrel cortex are synchronously activated by whisker input[7]. The spatial and temporal patterns of neurons and astrocytes are similar in response to whisker stimuli (Figure 3, 4). We further examined whether neurons and astrocytes in barrel networks coordinately encoded the sensory adaptation.

Figure 4d illustrates the cross-correlations of cellular events between the neurons and astrocytes. Each pixel in the matrices of Figure 4d represents the peak cross-correlation for a pair of neuron-astrocytes, and dark-red pixels present the best correlation, or vice versa. Correlation coefficients in central peaks averaged from seven experiments decrease from (0.31±0.03) for stimulus one to (0.19±0.03) for two ($P < 0.01$, Figure 4e). Less synchrony in response to identical stimuli indicates that the neurons and astrocytes in barrel networks coordinately encode the sensory adaptation.

2.5 The dynamics of functional connections among nerve cells is involved in encoding sensory adaptation

Mechanism for barrel cortical neurons and astrocytes coordinately to encode sensory adaptation is likely based on the dynamical changes of their functional connections. If it is a case, we expect to see that functional connections among barrel nerve cells are downregulated in response to identical stimuli. Functional connections among nerve cells were estimated by using their cross-correlations [18]. Correlation coefficients during spontaneous activities at these nerve cells were defined as no functional connection among them[7]. The averaged values above these correlation coefficients plus two-time standard deviations were defined as the functional connections[7]. It's worth mentioning that the layer Ⅱ/Ⅲ neurons in barrel cortex receive and intergrate complex inputs and their sychrony decreased with increasing spatial distance [22], the higher activity synchrony may not induced by a common inputs. Based on these principles, we analyzed functional connections among barrel cortical neurons and astrocytes in response to identical stimuli.

The astrocytes in the cortical are tightly connected with gap junctions, The synchrony is regulated by the activity of gap junctions, while it shows no evident the astrocytes would loss connection[14]. Based on this, we only analyzed the neuronal functional connections and neuro-astrocytic connections. Figure 5 illustrates functional connections among the neurons in response. The percentages of the functionally connected neurons in barrel cortex are 100 % in R1, compared with(95±2)% in R2(Figure 5b, $P < 0.05$). This result indicates that the neurons in barrel cortex are less functionally connected in response to the identical stimuli. The percentages of functional connections for a neuron with others in R1 and R2 are (76.9±7.1)% and (53.1±7)%, respectively (Figure 5c, $P < 0.05$). This result indicates that each barrel neuron is less functionally connected with other neurons in response to identical input frequency. Thus, the barrel cortical neurons encode neural signals for sensory adaptation through weakening their functional connections.

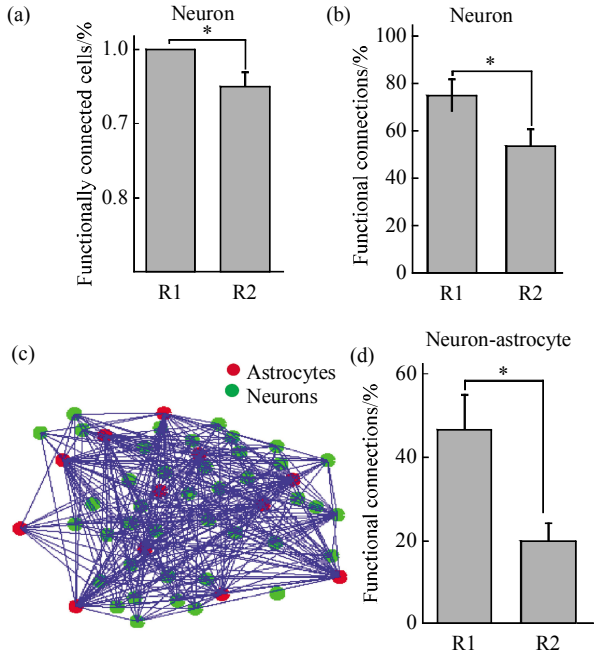

Fig. 5 Functional connections among barrel cortical cells decrease in response to identical paired burst-stimuli

(a) The percentages of functionally connected neurons in barrel network during R1 and R2. (b) The portion of functional connections for each neuron with others in R1 and R2. *$P < 0.05$ in student's t-test. (c) The functional connections (blue lines) between neurons (dark-green symbols) and astrocytes (dark-red). (d) The percentage of functional connections between neurons and astrocytes in R1 and R2. *$P < 0.05$ in student's t-test.

We also analyzed the functional connections between the neurons and astrocytes in response to identical paired burst-stimuli. Figure 5c illustrates the functional connections (blue lines) between the neurons (dark-green symbols) and astrocytes (red). The percentages of functional connections between neurons and astrocytes in R1 and R2 are (46.3±10.2)% and (19.4±4.9)%, respectively (Figure 5c, $P < 0.05$). Thus, the neurons and astrocytes in barrel cortex coordinately encode neural signals for sensory adaptation *via* weakening their functional connections.

2.6 AMPA receptor desensitization mediates weakened functional connections during sensory

In terms of molecular mechanisms underlying less functional connection among barrel cortical neurons and astrocytes during sensory adaptation, we hypothesized that the desensitization of synaptic receptors was involved in the weakening of functional connections among nerve cells. This hypothesis is based on a fact that a desensitization of AMPA-type glutamate receptors leads to synaptic depression [23–24]. Cyclothiazide (CTZ), an inhibitor of AMPA receptor desensitization [25], was selected to test this hypothesis. Two-photon cellular Ca^{2+} signals in response to whisker tactile input were analyzed to show the activity patterns of neurons and astrocytes in barrel cortex before and after using CTZ (**Materials and methods**).

Figure 6 illustrates the influences of cyclothiazide on the activity patterns, correlation coefficients and functional connections of the barrel cortical neurons and astrocytes in response to identical paired burst-stimuli. CTZ (10 μmol/L) induced acute and robust calcium response and fell back to the baseline. Whether CTZ changed the intrinsic property of the neural cells? We firstly measured the 1st response before and after CTZ effect as control, the calcium amplitudes show no significant changes. (before drug: (22.4±2.1)%, after drug: (21.7±3.6%), $P > 0.05$, t-test). CTZ appears to change a decrement pattern toward an increment (Figure 6a). The portions of increment, decrement and parallel in the neurons are (22.71±2.37)%, (63.67±2.93)% and (13.59±1.99)% under the controls, as well as are (47±5)%, (39±6)% and (14±4)% after CTZ application (n =5; Figure 6b). The portions of increment, decrement and parallel in the astrocytes are (25.82±2.27)%, (60.41±3.49)% and (13.77±2.29)% under the control, as well as are (45.57±4.43)%, (32.62±3.59)% and (22.15±4.07)% after using CTZ (Figure 6c). The correlation coefficients for neuron-astrocyte pairs are (0.30±0.02) and (0.20±0.02) in response to stimulus one and two under the control (Figure 6d; $P < 0.05$), as well as are (0.32 ± 0.04) and (0.35 ± 0.05) after CTZ application (P =0.4). CTZ also improves functional connections between neurons and astrocytes. The portions of their functional connections are (43.1±6.8)% in R1 and (21.3±4.5)% in R2 ($P < 0.01$; Figure 6e) under controls, and these values (48.4±5.4)% in R1 and

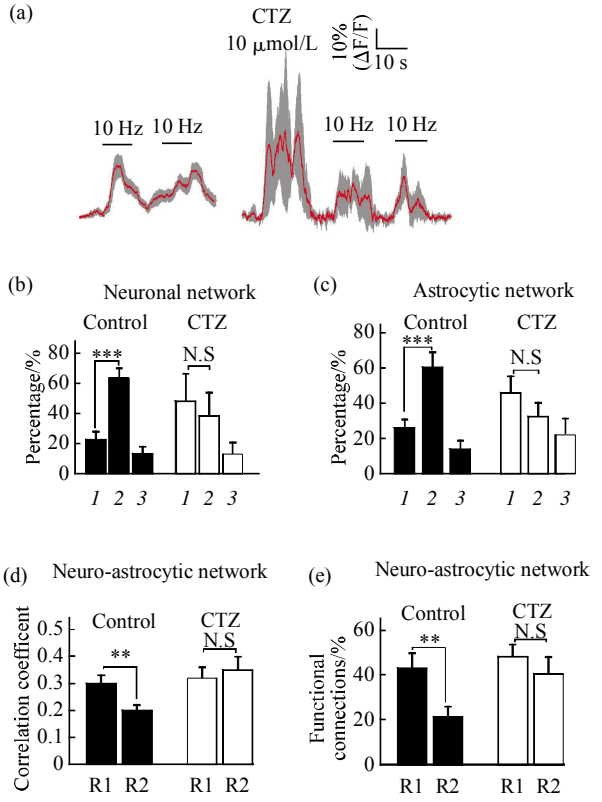

Fig. 6 The effects of cyclothiazide on the activity strength, synchrony and functional connections of barrel cortical neurons and astrocytes

10 μmol/L CTZ was locally applied onto the surface of barrel cortex. (a) Red trace shows sequential Ca^{2+} signals averaged from twenty neurons (gray traces) before and after using CTZ. (b) The percentages of decrement (R1>R2), increment (R1<R2) and parallel (R1 =R2) among the neurons (n=7) in response to the paired identical stimuli under the control (black bars) and CTZ application (whites). (c) Illustrates the percentages of decrement (R1>R2), increment (R1<R2) and parallel (R1 =R2) among the astrocytes (n=7) in response to the paired identical stimuli under the control (black bars) and CTZ application (whites). *1*: R1<R2; *2*: R1>R2; *3*: R1 =R2. (d) The comparison of averaged correlation coefficients for all active pairs of neuron-astrocytes in response to the paired identical stimuli under the conditions of control (black bars) and after using CTZ (whites). (e) The percentages of functional connections between neurons and astrocytes during R1 and R2 under the conditions of control (black bars) and after using CTZ (whites). *$P < 0.05$, and **$P < 0.01$ in student's t-test.

(40.6±7.5)% in R2 (P > 0.05) after CTZ use. These results indicate that the desensitization of AMPA receptors mediates activity decrement and asynchrony as well as functional connections in barrel cortical neurons and astrocytes for encoding sensory adaptation.

In addition, we studied the influence of CTZ on the adaptation of whisker tactile input in mice. As showed in Figure 7, the retraction durations of the whiskers under the control are (2.8±0.5) and (1.3±0.3) seconds in R1 and R2, respectively (P < 0.01, n=6). The retraction durations after CTZ use to barrel cortex are (2.4±0.2) and (2.2±0.1) seconds in R1 and R2, respectively. The data indicates that an inhibition of AMPA receptor desensitization improves the sensory adaptation in mice. It is noteworthy that in Figure 6 we found the inhibition of AMPA receptor desensitization block the decrement of functional connectivity in neuro-astrocytic network. Together with the data in Figure 7, it indicates AMPA receptor desensitization involves in regulation of cortical network connection in the process of somatosensory adaptation.

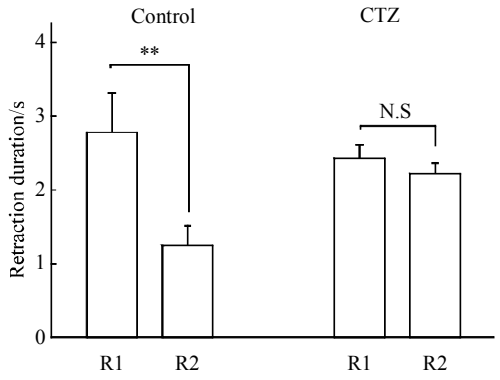

Fig. 7 The effects of cyclothiazide on the retraction duration of the mouse whiskers in reposnes to the paired identical stimuli

10 μmol/L CTZ was locally applied onto the surface of barrel cortex. Left shows the whisker retraction durations in response one and two under the control. *P < 0.05. Right shows the whisker retraction durations in response one and two after CTZ application.

3 Discussion

The mice demonstrate an adaptation of their whiskers to tactile stimuli in identical features(Figure 1). A population of neurons in barrel cortex is involved in encoding this sensory adaptation (Figure 2), which the neurons and astrocytes encode by decreasing their activity strength and synchrony (Figure 3, 4). The decreased functional connections among the neurons or astrocytes in sensory adaptation (Figure 5) are caused by the desensitization of AMPA-type glutamate receptors (Figure 6). In addition, the inhibition of AMPA receptor desensitization improves the sensory adaptation (Figure 7). Therefore, the neurons and astrocytes in barrel cortex coordinately encode the neural signals for sensory adaptation through AMPA receptor desensitization. Our studies strengthen a hypothesis that the sensory cortices play an important role in the sensory adaptation .

In the sensory systems, the stimulations in identical features induce the adaptation of sensations. The ability of producing the spikes at individual neurons was altered in sensory adaptation and sensitization [6, 26]. How the neurons and astrocytes integrated into neural networks encode these processes remains unclear. By studying the responses of the neurons and astrocytes to identical stimuli with electrophysiology, cellular imaging and pharmacology, we reveal that the barrel cortical neurons and astrocytes encode the sensory adaptation through lowering their activity strength, synchrony and functional connections mediated by AMPA receptor desensitization. Taken these data with a study that they are functionally upregulated in response to the increase of stimulus frequency[7], we suggest that the changes in functional connections among the neurons and astrocytes are basic forms to encode neural messages for sensory adaptation versus sensitization. Their conversions are associated with the alternation of AMPA receptor sensitivity in glutamatergic synaptic transmission.

The astrocytes are presumably to provide a micro-environment for the neurons to be functional[27-28]. The evidences included that a sensory stimulation altered Ca^{2+} signal in the astrocytes[7, 29], and that the astrocytes coupled with the neurons to mapping signals in visual cortex [30]. Our studies demonstrate that spatial and temporal activities are parallel between neurons and astrocytes (Figure 3, 4 and 6). Moreover, when the stimulus frequency rises or does not change, functional connections between the neurons and astrocytes increase or decrease correspondently (Figure 5 and reference [7]). These results update the roles of the astrocytes in the central nervous system, *i.e.*, they work with the neurons to encode neuronal signals.

There were no ideal experimental approaches directly to show functional connections among the neurons and astrocytes in neural network as well as their role in brain functions, though two-photon cellular imaging and electrode arrays were used to study the activities of multiple neurons. However, the analyses of activity synchrony and cross-correlations among nerve cells, such as cellular Ca^{2+} signal events, provided a potential revenue for estimating the functional connections among these nerve cells [7]. We combined the analyses of cross-correlation among neurons and astrocytes with the effect of glutamatergic synaptic transmission. A fact that the attenuation of AMPA receptor desensitization to increase neuronal functional connections through the synapses is associated with the upregulation of cross-correlation (Figure 6) strengthens the use of cross-correlation to estimate functional connections. Hence, the investigation of functional connectivity among neurons and astrocytes enable us to understand the cellular network basis of sensory processing, such as adaptation.

Although AMPA receptor desensitization in glutamatergic synapses are involved in the activity decrement, asynchrony, less functional connection among the nerve cells for sensory adaptation, other types of cellular communications, such as transmitter release, GABAergic synapses and gap junction, cannot be ruled out from sensory adaptation. For instance, the sensory inputs innervate to inhibitory neurons in barrel cortex. Their activations during stimulus one may inhibit the activity of excitatory neurons, so that the second responses are attenuated. The changes in the balance between the excitation and inhibition as well as the GABAergic inhibition shaped frequency adaptation and adaptation in thalamic barreloid [31]. These possibilities would be tested in the future studies.

It is noteworthy that the inhibition of AMPA receptor desensitization in barrel cortices by CTX removes sensory adaptation (Figure 7). The action sites of CTZ may be the synapses from the thalamus to the barrel cortex and from the barrel cortex to whisker-controlling motor cortex. This effect may also be due to a possibility that CTZ application affects the descending pathways from the cortex to the low levels of sensory afferent nerves. These possibilities will be examined in our future studies.

Previous studies indicate that mGluR mediate the interaction between synaptic transmission and astrocytic calcium responses in the cortical information processing [29–30]. We found the increased synchronization and connections between neurons and astrocytes after AMPA receptor desensitization was removed (Figure 6). It indicates that the increase of synaptic transmission improves the neuronal network glutamate cycling which likely enhances the communication between neurons and astrocytes through the mGluR and ionotropic GluR as well.

The astrocytic calcium signal is complex in the activated brain local networks [14]. Besides neural transmission, the calcium signals in the astrocytes are tightly coupling to their hemodynamics and metabolism [14]. Some astrocytes also show robust spontaneous calcium signals and not coupling with evoked neuron activity [32]. It is predictable that the asynchrony and functional connection in local neuronal network induced by AMPA receptor desensitization reduce the communication to astrocytic network and thus some astrocytes show sensory stimuli uncoupling calcium signals.

In conclusion, by studying the activity patterns of barrel cortical nerve cells and the sensitivity of AMPA-type glutamate receptors in response to the paired identical stimuli, we demonstrate that the neurons and astrocytes in barrel cortex coordinately encode neural signals for the sensory adaptation *via* decreasing their functional connections mediated by AMPA receptor desensitization.

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AMPA 受体介导的神经元和星形胶质细胞网络编程小鼠胡须感觉适应 *

赵　君[1, 2)]　王晋辉[1, 2)**]

([1)] 中国科学院生物物理研究所，脑与认知国家重点实验室，北京 100101；[2)] 中国科学院大学，北京 100049)

摘要　动物感觉输入的适应性影响了它们对外界环境改变的意识和反应．感觉通路各层次，诸如感受器、传入神经和中枢系统等，反应活性的降低可能与感觉适应性相关联．在感觉适应过程中，皮层局部网络中神经元和星形胶质细胞对信号的编程机制仍有待进一步研究．利用活体双光子成像、电生理记录即药理学方法，我们分析了小鼠 barrel 皮层神经元和星形胶质应答重复的胡须感觉输入动力学．相同特征的胡须感觉刺激诱发了神经元和星形胶质细胞反应活性的降低，并且它们的活动在空间上和时间上去同步化，神经元和星形胶质细胞之间的缺少协调性．这种神经元和星形胶质细胞功能在空间和时间性质上的下调被局部施加 AMPA 受体脱敏感抑制剂所逆转．因此，在胡须感觉适应过程中，barrel 皮层神经元和星形胶质细胞反应活性的下降和去同步化是由 AMPA 受体脱敏感参与介导完成的．

关键词　神经网络，星形胶质细胞，桶状皮层，胡须，适应性，AMPA 受体，活体双光子成像

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** 通讯联系人．

Tel: 010-64888472, E-mail: jhw@sun5.ibp.ac.cn

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