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Optical analysis of acute changes after peripheral nerve injury in spatio-temporal pattern of neural response to forelimb stimulation in rat somatosensory cortex

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Abstract

Peripheral nerve injury induces functional reorganization of the central nervous system. The mechanisms underlying this reorganization have been widely studied. Our previous study involving multiple-site optical recording reported that a neural excitatory wave induced by somatic stimulation begins in a small area and propagates in the cortex. In the present study, to examine the possible role of this propagation wave in cortical reorganization, we analyzed the early changes in the spatio-temporal pattern of the sensory-evoked wave immediately, and 30 min, after nerve injury. The response to hypothenar stimulation, innervated by the ulnar nerve and adjoining the median nerve area, persisted after injury to either the ulnar or median nerve. Initially, we assessed changes in the response pattern at the focus. The latency increased after ulnar nerve injury, whereas no change was observed after median nerve injury. Similarly, no change was noted in the duration of the response signal with either nerve injury. Second, changes in the propagation wave pattern were analyzed. Ulnar nerve injury decreased the propagation velocity in the medial direction but the median nerve injury induced no changes. These results indicated that the propagation wave pattern is readily altered, even immediately after nerve injury, and suggest that this immediate change in the spatio-temporal pattern is one of the factors contributing to the cortical reorganization.

1. Introduction

Loss of sensory input to the central nervous system, induced by limb severance as well as peripheral nerve or spinal cord injury, reorganizes the

somatosensory cortex; the lost function is then either recovered or compensated for. This reorganization consists of at least two phases (Florence et al., 1997): a rapid one (minutes) and a slow one (days to weeks). The former occurs immediately after peripheral nerve injury; the cortical area corresponding to the nearby body regions expands, but is limited, to the "silent area" (Merzenich et al., 1983; Calford and Tweedale, 1988; Cusick et al., 1990). This expansion is triggered by the unmasking of input that is normally depressed by the input via injured nerve (Jain et al., 1998). This immediate change in the sensory response appears to play a crucial role in inducing the second phase of the reorganization of the central nervous system (Jain et al., 1998).

Many previous studies on the rapid phase have focused on the change in response of neurons within a restricted area. However, the rapid phase is not a local phenomenon that is limited to the small cortical area somatotopically corresponding to the denervated body part; rather, it apparently extends to the entire somatosensory cortex. It was demonstrated that responses of the forepaw cortex to forepaw stimulation increases immediately after thoracic (T9-T10) spinal cord transection (Aguilar et al., 2010). Furthermore, this immediate increase in response was observed after peripheral denervation of the hindpaw (Humanes-Valera et al., 2014). They discussed the horizontal progression of neuronal activity through corticocortical connections as one of the possible mechanisms.

With respect to horizontal interactions of neuronal activity in the cortex, the propagation wave was demonstrated by introducing optical recording with a voltage-sensitive dye (VSD) based on direct measurement of

membrane potentials (Cohen and Salzberg, 1978). Using multiple-site optical recording with high temporal resolution, it has been reported that neural activity elicited by sensory stimulation is not limited to the somatotopically corresponding site, but, rather, is propagated widely through the cortical area in waves (Petersen et al., 2003a; Tucker and Katz, 2003; Roland et al., 2006; Song et al., 2006; Wu et al., 2008; Morales-Botello et al., 2012; Wester and Contreras, 2012). This propagation wave slightly depolarizes neurons, and thereby alters their responses to subsequent input (Wu et al., 2008), which may play a role in the rapid phase of reorganization.

Previously, we found that the wave propagating pattern in the somatosensory cortex is affected by the condition of sensory stimulation: when the right hindpaw and forepaw were stimulated at the same time, the evoked two waves in the left cortex interacted with each other in different ways, depending on the time difference between the stimulations, and accordingly, the sensory response to the stimulation was either depressed or enhanced (Hama et al., 2015, 2018). Thus, the wave is susceptible to the conditions of sensory input, and we hypothesized that the propagating pattern of the wave as well as neuronal responses is modified immediately after denervation, which suddenly deprives the cortex of sensory input from a certain body site. This may alter the cortical neuron activity to peripheral stimulation to the out-of-denervation area, and, as a constituting process of the rapid phase, contribute to the development of the slow phase of reorganization. To date, the effect of peripheral nerve injury on the propagating pattern of wave is unknown. As the first step, we analyzed the immediate effect of median or ulnar nerve injury on the pattern of wave

propagation in the rat somatosensory cortex, induced by stimulation to the hypothenar pad, which is located within the median nerve territory adjoining the ulnar nerve territory.

2. Experimental Procedure

Animals

We used 20 young adult female rats (11-13 weeks old and 240-310 g). All animals were purchased commercially (CLEA Japan Inc., Tokyo Japan) and kept under a 12:12-h light-dark cycle, with food and water available *ad libitum*. Animals were anesthetized by the intraperitoneal injection of a mixture of Urethan (800 mg/kg) and α-Chloralose (80 mg/kg). During the experiment, the depth of anesthesia was monitored using heart rate and electrocorticographic (ECoG) signals and maintained at stage III-3 or 4. Supplemental anesthetic (5% of initial dose) was given via a cannula placed in the femoral vein as necessary. The body temperature was maintained at 35 ºC with a disposable heating pad. All animal experiments were performed in compliance with the Guidelines for Animal Experimentation of the Center for Integrated Research in Science, Shimane University.

Nerve injury

Animals were divided into three groups: ulnar nerve injury (Ui, n = 7), median nerve injury (Mi, n = 7), and Sham-operation (Sa, n = 6). For each group, the ulnar and median nerves of the right forelimb were exposed by incising the skin covering these nerves. These procedures were done before creating the craniotomy window. Just before optical recording, the median or

ulnar nerve was pinched with artery forceps for 10 s at the wrist (cross marks in Fig. 1A). We preliminarily confirmed that this procedure completely blocked the conduction of an action potential. The Sa group underwent the same surgical procedure without nerve pinching.

Optical recording

The left somatosensory cortex was exposed and stained with VSD (RH414; Molecular Probes; 0.4 mg/mL)(Orbach et al., 1985). A neural response was induced by electrical stimulation (0.5 ms, 1 mA) on the hypothenar pad of the right forelimb using a pair of tungsten needles. A 1024-element photo diode array was used to detect fluorescence from a magnified image of the stained cortex. We created a craniotomy window to fit the semi-circular shape plano-concave lens that was used to improve the quality of optical recording (Kawai et al., 2014). Approximately 450 elements in the photodiode array covered the craniotomy window and we recorded optical signals simultaneously from these elements (indicated as "recording area" in Fig. 1). The size of one photodiode pixel and the pixel interval corresponded to 225 × 225 μm and 250 μm on the preparation, respectively. The electrocardiogram (ECG) was recorded simultaneously with the optical measurement, and used to reduce the pulsation artifact contained in the optical signal (Hama et al., 2010). The detailed methods of our system for multiple-site optical recording of neural activity and noise reduction were described in previous papers (Hirota et al., 1995; Hirota and Ito, 2006; Hama et al., 2010, 2015, 2018; Kawai et al., 2014). We recorded optical signals for 60 s continuously starting at 5 min before, immediately after (starting within

1 minute after injury), and 30 min after nerve injury (Fig. 1C). The hypothenar was stimulated 100 ms after the initiation time of the R wave in the ECG recording. To avoid frequent stimulation, the stimulation was given at every sixteenth heartbeat; corresponding to the interstimulus interval of approximately 2 s (Hama et al., 2010). Although the signal-to-noise ratio of the optical signal recorded by our system was sufficiently high to analyze in a single sweep, 6 to 26 trials were averaged in order to eliminate trial-to-trial variability. Only trials indicating no spontaneous activity 50 ms before stimulation (see Fig. 1D) were used to obtain the average, and we used the averaged data for analyses (Hama et al., 2015).

Data analysis

Each optical signal size was converted to a fractional change (ΔF/F), that is, a change in fluorescence intensity (ΔF) divided by the background intensity (F). The baseline level of the optical signal was established as the average value during the 100 ms before stimulation. The peak amplitude was defined as the maximal difference in the value from the baseline in each signal. The duration of the signal was defined as the full width at half amplitude of the response signal. The latency was defined as the time from the stimulus onset to half amplitude of the response signal. The pixel with the shortest latency was defined as the focus. The average propagation velocity from the focus to each pixel was calculated by dividing the pixel distance from the focus by the difference between the latencies. All data in the figures are shown as the mean ± SD. We used the paired t-test and

adjusted p-values using the Holm-Bonferroni method for multiple comparisons. $P < 0.05$ was considered to be "suggestive".

3. Results

3.1 Characteristics of cortical neural response evoked by hypothenar pad stimulation

Figure 2A shows multiple-site optical recording of the neural response induced by hypothenar pad stimulation in an animal before injury (PRE). The neural response optical signals were detected from the entire region of the recording area. Although they appeared synchronized over the whole area, there were slight differences in the latency of the signal among pixels at a higher sweep speed (Fig. 2B). To visualize the propagating pattern of neural activity, we constructed an isochrone map based on the differences among latencies at each pixel (Fig. 2C). The neural response signal first appeared at approximately 20 ms after stimulation in the left-bottom area of the recording (pixel labeled *b* in Fig. 2A), corresponding to the forelimb area in the somatotopic map and propagated to the entire recording area. The response signal of our optical recording reflects the response neural activity within shallower layers, while the somatic evoked potential reflects the neural activity that within the deeper layers, as well (Hama et al., 2015). Therefore, the latency of our response signal should be longer than that of the somatic evoked potential. On viewing Fig. 2C, the density of isochrone curves was higher in the postero-medial direction than that in the medial or posterior direction, that is, the propagation velocity in the postero-medial direction was

relatively slower. The propagation pattern was non-uniform,-showing a weak directional preference.

3.2 Changes in neural response at the focus

Figure 3 shows typical examples of the multiple-site optical recording of immediately after (Ui0) and 30 min after (Ui30) ulnar nerve injury. Figure 2 corresponds to the PRE recording for this example. In the example, neural responses persisted in the presence of nerve injury and spread over the entire recording area as before (Fig. 3 A and B). The median nerve injury also failed to abolish the neural response (data not shown).

We analyzed the neural responses at the focus (Fig. 3C and D). The peak amplitude decreased 30 min after either ulnar or median nerve injury, but the latency increased only after ulnar nerve injury. Statistical comparison revealed that the decrease in the response amplitude after the nerve injury was suggestive (Fig. 4A). However, since the signal size in the Sa group also tended to decrease, the decrease in signal size after nerve injury was attributed to a signal degradation, not due to a physiological factor (see Discussion). To compensate for the effect of the signal degradation, we normalized the peak amplitude to the amplitude at PRE (right panel in Fig. 3C and D). After nerve injury, the response latency suggestively increased in the Ui group (PRE: 23.4 ± 2.7 ms, Ui0: 26.4 ± 4.2, Ui30: 27.6 ± 3.9 ms) but not in Mi group (PRE: 28.8 ± 5.0, Mi0: 29.0 ± 5.3 ms, Mi30: 27.6 ± 5.5 ms) (Fig. 4B). The change in the response latency was not suggestive in the Sa group (Fig. 4B). To clarify whether the nerve injury affected the time course of the response signal itself, we evaluated changes in the signal duration. Again, no

suggestive change was observed (Fig. 4C). Thus, the ulnar nerve injury increased the response latency, but did not induce other changes in the time course of neural responses at the focus.

3.3 Changes in the propagation pattern

The propagation wave should determine when and where the neuron can respond to input, possibly being one of the important functions of the propagation wave. We analyzed the changes in the propagation pattern after nerve injury within 30 min. After nerve injury, the overall propagating pattern was similar to that before injury (Fig. 5). However, the density of isochrone in Ui became higher (Fig. 5A), indicating that the propagation velocity became slower. To examined differences in the propagation velocity of the wave among the directions of propagation, we measured the velocity in four directions: antero-medial, postero-medial, antero-lateral, and postero-lateral directions (Fig. 6). The propagation velocity before injury differed among these directions: the antero-lateral direction was the fastest (212.3 ± 54.3 μm/ms) and antero-medial direction was the slowest (138.2 ± 35.9 μm/ms). In the Ui group, the velocity in the antero-medial direction suggestively decreased 30 min after nerve injury (111.2 ± 22.5 μm/ms). The velocity in the postero-medial direction (PRE: 164.5 ± 46.5 μm/ms) also decreased, it occurred at Ui0 (90.1 ± 33.7 μm/ms) and was maintained at least until Ui30 (88.4 ± 13.3 μm/ms, Fig. 6A and B). There was no suggestive change in antero- or postero-lateral directions (Fig. 6C and D). The slowest direction changed from antero-medial to postero-medial. In Mi and Sa groups, there was no suggestive change in the velocity in any direction (Fig. 6). Thus,

the propagating pattern was altered by ulnar nerve injury but not median nerve injury.

4. Discussion

Using our multiple-site optical recording system with VSD, we demonstrated, for the first time, the immediate changes in the spatio-temporal pattern of neural activity in the somatosensory cortex after peripheral nerve injury. We compared the hypothenar-induced cortical responses before, immediately after, and 30 min after ulnar or median nerve injury. Although the response persisted after ulnar or median nerve injury, the influence of ulnar nerve injury was more marked than that of median nerve injury. Ulnar nerve injury increased the latency at the focus and altered the propagation pattern. However, median nerve injury had no effect on neural response.

4.1 Spatio-temporal pattern of neural activity in the somatosensory cortex

It is well known that peripheral nerve injury induces the reorganization of the somatosensory cortex to compensate for the lost function. This reorganization consists of two phases (Florence et al., 1997). The rapid phase, on the one hand, is as follows: the parts of injured nerve territory in the somatosensory cortex are activated by the input from nearby body regions (Merzenich et al., 1983; Calford and Tweedale, 1988; Cusick et al., 1990) and occurs within minutes to hours after peripheral nerve injury. This expansion is caused by the unmasking of input which is normally depressed by the input via the injured nerve (Jain et al., 1998). On the other hand, the changes

observed in the slow phase involve the reorganization of topographic order (Kaas et al., 1983). The structural reorganization, that is, axonal sprouting, participates in this process (Florence and Kaas, 1995). Since the rapid phase may influence the slow phase (Jain et al., 1998), it is important to study the changes in the neuronal response to sensory input immediately after the nerve injury.

Studies on the rapid phase have focused on the changes in the neuronal activity within the restricted region, the activity of which mainly reflects the subcortical input. However, a vast number of cortical neurons are synaptically interconnected, and form a highly complex network. Therefore, the sensory information into neurons within the restricted area affects the neuronal activity in a wider cortical area. This process would partially be realized, as multiple-site optical recording has revealed, in the form of a "propagation wave", which begins at the focus, and propagates while depolarizing cortical neurons, thereby altering their responsiveness to sensory input. If we refer to the probability of response of individual cortical neurons to input with a phrase such as "the state of the cortical network" for short, then, we may say simply that propagation wave affects the state of the cortical network.

Sensory deprivation could also affect the state of the cortical network. Peripheral nerve injury immediately affects the neural response in the wider somatosensory cortex. Humanes-Valera et al. (2014) found that the response of neurons within the forepaw cortex to forepaw stimulation increases immediately after hindlimb denervation. Since the input remained the same, the changes in the response would be attributed to the changes in the state

of the cortical network. The state of the cortical network, susceptible to spontaneous as well as evoked activity from moment to moment (Petersen et al., 2003b; Gao et al., 2012; Hama et al., 2015, 2018), is likely to have been altered after peripheral nerve injury, causing marked changes in the ascending neural activity.

The propagation wave, closely related to the state of the cortical network, is also affected by peripheral nerve injury. In the present study, we found that the pattern of propagation wave changed immediately after peripheral nerve injury. These findings, together with those by Humanes-Valera et al. (2014), could be considered as one of the earliest manifestations of the cortical reorganization. This alteration of the propagation wave would may serve as a factor in cortical reorganization.

4.2 Neural responses after nerve injury

In this study, we analyzed the neural response induced by hypothenar stimulation. The hypothenar is located within the ulnar nerve area and adjoins the median nerve area (Waters et al., 1995; Li et al., 2013). Persisting responses after ulnar nerve injury (Fig. 3C) indicate extension of the stimulated skin area to the median nerve area. In the pre-injury control, the stimulation to the hypothenar should have induced responses in the cortical median nerve area as well as in the ulnar nerve area, although we were unable to observe the corresponding difference in the focus location after ulnar or median nerve injury, probably due to the insufficient spatial resolution of our system (250-μm in pixel intervals). In addition, the input to the sensory cortex via the median nerve arrived later than that via the ulnar

nerve (Fig. 4B). This delay should be caused by the longer latency of undominant input from neighbors (Schroeder et al., 1995; Li and Waters, 1996). The peak amplitude of response decreased after nerve injury (Fig. 4A). However, since the signal size in the Sa group also tended to decrease, the decrease in signal size following nerve injury was unlikely to be caused by a physiological factor. Indeed, signal size reduction in the long-term optical recording using VSDs has often been reported and ascribed to non-specific causes such as dye bleaching (Momose-Sato et al., 1995; Lippert et al., 2007; Takagaki et al., 2008).

In addition, the spatio-temporal pattern of the neural response was not altered by median nerve injury (Fig. 5B and 6). These results show that the median nerve-mediated response is suppressed, and does not influence the spatio-temporal pattern of the response to hypothenar stimulation before ulnar nerve injury. Both subcortical and cortical structures may contribute to this suppression. Particularly, the propagation wave suppresses the occurrence of the subsequent wave (Civillico and Contreras, 2006; Gao et al., 2012; Hama et al., 2018), and thus, the median nerve-mediated response should have been suppressed by the preceding depolarization, owing to the excitatory wave mediated by the ulnar nerve. All peripheral nerves have "neighbors", that may be activated by the same stimulus and send sensory information, slightly different, to the cortex in parallel. Malfunction of one nerve branch allows a neighbor branch-evoked propagation wave, which is normally hidden, to emerge. Thus, what we observed in the hypothenar-representing cortex could occur anywhere in the sensory cortex.

4.3 Possible contribution of the changes in the spatio-temporal pattern to reorganization of the somatosensory map

Sensory deprivation results in expansion of the cortical area corresponding to the adjacent body part (Doetsch et al., 1996; Jain et al., 1998; Feldman and Brecht, 2005; Higley and Contreras, 2005). In adult animals, map reorganization occurs only in layers 2/3 and 5 (Diamond et al., 1994). Since the spike timing-dependent plasticity is crucial for the cortical plasticity (Feldman, 2012), such synaptic changes are expected in-layers 2/3 and 5 for cortical reorganization. Indeed, principal whisker deprivation results in the depression of synapse from layer 4 to layers 2/3 neurons by reversing the firing order of layers 4 and 2/3, and reducing the firing correlation between layer 4 and 2/3 neurons (Allen et al., 2003; Celikel et al., 2004). Thus, the spiking probability of layer 2/3 neurons in the presence of sensory stimulation may be important for cortical reorganization.

The propagating excitatory wave spreads in layers 2/3 and, by slightly depolarizing, alters the firing probability of layers 2/3 neurons in response to sensory input, and thus, should determine when and where the neuron can respond to input (Civillico and Contreras, 2006; Wu et al., 2008; Wester and Contreras, 2012; Hama et al., 2018). In this study, we found that the ulnar nerve injury immediately induced that change in the propagation pattern of neural activity. The propagation velocity of the excitatory wave decreased in a medial direction; the direction to the cortex associated with the ulnar nerve (Figs. 5 and 6). It is suggested that ulnar nerve injury, by changing the pattern of the propagating wave, affects the response behavior of layer 2/3 neurons in the ulnar nerve-representing cortex to sensory input via the

median nerve, which innervates surrounding body parts. As discussed above, the changes in the propagation pattern immediately after nerve injury do not appear to be specific to the present finger region; rather, this propagation wave-related modulation of the cortical neurons is likely to occur generally in the sensory cortex. The changes in the propagation wave pattern may be one of the essential occurrences in the reorganization of the sensory map. Further study is required to investigate the effect of modifying the propagating pattern on the somatotopic map and the changes in the propagation pattern during reorganization of the sensory map.

4.4 Limitations

In this study, we used only female animals. Sexual difference was reported in the gene expression pattern of rats 24 hours after infraorbital nerve transection (Orczyk et al., 2017). The changes in the propagation wave may also differ between females and males immediately after peripheral nerve injury.

Peripheral nerve injury affects the neuronal activity of the thalamus and the brainstem nuclei (Florence et al., 1997). The pattern of the propagation wave may be affected by the activity of those neurons. Further study is needed to clarify the contribution of the change in subcortical structures on the propagation wave.

4.5 Concluding remarks

We demonstrated changes in the propagating wave pattern immediately after peripheral nerve injury. Since the propagating wave affects

the neuronal activity, and changes in the neuronal response to sensory stimulation may influence the following cortical reorganization, it is suggested that the changes in the propagating pattern contribute to the cortical reorganization. Thus, our results provide a new perspective in the study of cortical reorganization.

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References

Aguilar J, Humanes-Valera D, Alonso-Calviño E, Yague JG, Moxon KA, Oliviero A, Foffani G (2010) Spinal cord injury immediately changes the state of the brain. J Neurosci 30:7528–7537.

Allen CB, Celikel T, Feldman DE (2003) Long-term depression induced by sensory deprivation during cortical map plasticity in vivo. Nat Neurosci 6:291–299.

Calford MB, Tweedale R (1988) Immediate and chronic changes in responses of somatosensory cortex in adult flying-fox after digit amputation. Nature 332:446–448.

Celikel T, Szostak VA, Feldman DE (2004) Modulation of spike timing by sensory deprivation during induction of cortical map plasticity. Nat Neurosci 7:534–541.

Civillico EF, Contreras D (2006) Integration of Evoked Responses in Supragranular Cortex Studied With Optical Recordings In Vivo. J Neurophysiol 96:336–351.

Cohen LB, Salzberg BM (1978) Optical Measurement of Membrane Potential. Rev Physiol Biochem Pharmacol 83:35–88.

Cusick CG, Wall JT, Whiting JHJ, Wiley RG (1990) Temporal progression of cortical reorganization following nerve injury. Brain Res 537:355–358.

Diamond ME, Huang W, Ebner FF (1994) Laminar comparison of somatosensory cortical plasticity. Science (80-) 265:1885–1888.

Doetsch GS, Harrison TA, MacDonald AC, Litaker MS (1996) Short-term plasticity in primary somatosensory cortex of the rat: rapid changes in magnitudes and latencies of neuronal responses following digit denervation. Exp Brain Res 112:505–512.

Feldman DE (2012) The Spike-Timing Dependence of Plasticity. Neuron 75:556–571.

Feldman DE, Brecht M (2005) Map Plasticity in Somatosensory Cortex. Science (80-) 310:810–815.

Florence SL, Jain N, Kaas JH (1997) Plasticity of Somatosensory Cortex in Primates. Semin Neurosci 9:3–12.

Florence SL, Kaas JH (1995) Large-scale reorganization at multiple levels of the somatosensory pathway follows therapeutic amputation of the hand in monkeys. J Neurosci 15:8083–8095.

Gao X, Xu W, Wang Z, Takagaki K, Li B, Wu JY (2012) Interactions between two propagating waves in rat visual cortex. Neuroscience 216:57–69.

Grinvald A, Hildesheim R (2004) VSDI: A new era in functional imaging of cortical dynamics. Nat Rev Neurosci 5:874–885.

Hama N, Ito S-I, Hirota A (2010) An improved multiple-site optical membrane potential-recording system to obtain high-quality single sweep signals in intact rat cerebral cortex. J Neurosci Methods 194:73–80.

Hama N, Ito S-I, Hirota A (2015) Optical imaging of the propagation patterns of neural responses in the rat sensory cortex: Comparison under two different anesthetic conditions. Neuroscience 284:125–133.

Hama N, Kawai M, Ito S-I, Hirota A (2018) Optical study of interactions among propagation waves of neural excitation in the rat somatosensory cortex evoked by forelimb and hindlimb stimuli. J Neurophysiol 119:1934–1946.

Hama N, Kawai M, Ito S-I, Yoshida Y, Fujita Y, Hirota A (2019) Concave-shaped transparent electrode to simultaneously monitor electrical activity from multiple sites within the optical sampling area of the intact rat cerebral cortex. J Physiol Sci 69:543–551.

Higley MJ, Contreras D (2005) Integration of Synaptic Responses to Neighboring Whiskers in Rat Barrel Cortex In Vivo. J Neurophysiol 93:1920–1934.

Hirota A, Ito S-I (2006) A Long-Time, High Spatiotemporal Resolution Optical Recording System for Membrane Potential Activity via Real-Time Writing to the Hard Disk. J Physiol Sci 56:263–266.

Hirota A, Sato K, Momose-Sato Y, Sakai T, Kamino K (1995) A new simultaneous 1020-site optical recording system for monitoring neural activity using voltage-sensitive dyes. J Neurosci Methods 56:187–194.

Humanes-Valera D, Foffani G, Aguilar J (2014) Increased cortical responses to forepaw stimuli immediately after peripheral deafferentation of hindpaw inputs. Sci Rep 4:7278.

Jain N, Florence SL, Kaas JH (1998) Reorganization of somatosensory cortex after nerve and spinal cord injury. News Physiol Sci 13:143–149.

Kaas JH, Merzenich MM, Killackey HP (1983) The reorganization of somatosensory cortex following peripheral nerve damage in adult and developing mammals. Annu Rev Neurosci 6:325–356.

Kawai M, Hama N, Ito S-I, Hirota A (2014) Improvement of the optical imaging technique for intact rat brain using a plano-concave lens. J Physiol Sci 64:445–449.

Li CX, Waters RS (1996) In vivo intracellular recording and labeling of neurons in the forepaw barrel subfield (FBS) of rat somatosensory cortex: possible physiological and morphological substrates for reorganization. Neuroreport 7:2261–2272.

Li R, Hettinger PC, Machol JA, Liu X, Stephenson JB, Pawela CP, Yan J-G, Matloub HS, Hyde JS (2013) Cortical plasticity induced by different degrees of peripheral nerve injuries: a rat functional magnetic resonance imaging study under 9.4 Tesla. J Brachial Plex Peripher Nerve Inj 8:4.

Merzenich MM, Kaas JH, Wall J, Nelson RJ, Sur M, Felleman D (1983) Topographic reorganization of somatosensory cortical areas 3b and 1 in

adult monkeys following restricted deafferentation. Neuroscience 8:33–55.

Morales-Botello ML, Aguilar J, Foffani G (2012) Imaging the spatio-temporal dynamics of supragranular activity in the rat somatosensory cortex in response to stimulation of the paws. PLoS One 7:1–16.

Orbach HS, Cohen LB, Grinvald A (1985) Optical mapping of electrical activity in rat somatosensory and visual cortex. J Neurosci 5:1886–1895.

Orczyk JJ, Batka R, Gore A, Maio-Lexa M, Kulkarni A, Garraghty PE (2017) Female rat transcriptome response to infraorbital nerve transection differs from that of males: RNA-seq. J Comp Neurol 525:140–150.

Petersen CCH, Grinvald A, Sakmann B (2003a) Spatiotemporal Dynamics of Sensory Responses in Layer 2/3 of Rat Barrel Cortex Measured In Vivo by Voltage-Sensitive Dye Imaging Combined with Whole-Cell Voltage Recordings and Neuron Reconstructions. J Neurosci 23:1298–1309.

Petersen CCH, Hahn TTG, Mehta M, Grinvald A, Sakmann B (2003b) Interaction of sensory responses with spontaneous depolarization in layer 2/3 barrel cortex. Proc Natl Acad Sci 100:13638–13643.

Pinto DJ, Patrick SL, Huang WC, Connors BW (2005) Initiation, Propagation, and Termination of Epileptiform Activity in Rodent Neocortex In Vitro Involve Distinct Mechanisms. J Neurosci 25:8131–8140.

Roland PE, Hanazawa A, Undeman C, Eriksson D, Tompa T, Nakamura H, Valentiniene S, Ahmed B (2006) Cortical feedback depolarization waves: A mechanism of top-down influence on early visual areas. Proc Natl Acad Sci 103:12586–12591.

Schroeder CE, Seto S, Arezzo JC, Garraghty PE (1995) Electrophysiological evidence for overlapping dominant and latent inputs to somatosensory cortex in squirrel monkeys. J Neurophysiol 74:722–732.

Song WJ, Kawaguchi H, Totoki S, Inoue Y, Katura T, Maeda S, Inagaki S, Shirasawa H, Nishimura M (2006) Cortical intrinsic circuits can support activity propagation through an isofrequency strip of the guinea pig primary auditory cortex. Cereb Cortex 16:718–729.

Tanifuji M, Sugiyama T, Murase K (1994) Horizontal propagation of excitation in rat visual cortical slices revealed by optical imaging. Science (80-) 266:1057–1059.

Tucker TR, Katz LC (2003) Spatiotemporal Patterns of Excitation and Inhibition Evoked by the Horizontal Network in Layer 2/3 of Ferret Visual Cortex. J Neurophysiol 89:488–500.

Waters RS, Li CX, McCandlish CA (1995) Relationship between the organization of the forepaw barrel subfield and the representation of the forepaw in layer IV of rat somatosensory cortex. Exp Brain Res 103:183–197.

Wester JC, Contreras D (2012) Columnar Interactions Determine Horizontal Propagation of Recurrent Network Activity in Neocortex. J Neurosci 32:5454–5471.

Wu J-Y, Xiaoying H, Chuan Z (2008) Propagating Waves of Activity in the Neocortex: What They Are, What They Do. Neuroscientist 14:487–502.

Figure Legends

Figure 1 Experimental procedure

Location of the recording area within the rat neocortex. Upper panel: relative recording position. The circles drawn in dashed lines and-semicircle indicate the primary somatosensory area (SI) and craniotomy window, respectively. Bottom panel: enlargement of the craniotomy window. The black polygon indicates the recording area, which included hind- and forelimb areas (gray area). Within the somatotopic map, the area innervated by the ulnar nerve is indicated as the shaded area. D2 and D5 indicates the digits 2 and 5, respectively. (B) Positions of the stimulation site and nerve injury. The photograph shows the right forelimb after removing skin and muscles to expose the ulnar and median nerves. Ulnar or median nerve injury was induced at the wrist, indicated by the cross marks. The electrical stimulation was applied at the hypothenar pad shown by a circle using a pair of inserted tungsten needles. (C) Recording protocol. We conducted optical recording for 60 s continuously three times: starting 5 min before nerve injury (PRE), immediately after (Mi0 or Ui0), and 30 min after nerve injury (Mi30 or Ui30). The same timing was used for the Sham-operation group (PRE, Sa0 and Sa30). (D) An example of continuous optical recording. Vertical thin lines indicate timings of stimulation. Evoked neural activities (pointed with black or white arrowheads) and spontaneous ones were observed. Only trials pointed with black arrow head (with no spontaneous activity having occurred within 50 ms

before stimulation) were used for average; trials pointed with white arrow, preceded by a spontaneous activity, were omitted. In this and the following figures, optical signals are shown in a fractional change and calibration are given in the lower-right corner of optical signals by an arrow. The direction indicates a decrease in fluorescence (corresponding to depolarization of the membrane potential) and the length represents the stated value of the fractional change (ΔF/F). The vertical bar in the Heartbeat row is the initiation timing of R wave in the ECG recording.

Figure 2 An example of the neural response in the sensory cortex induced by hypothenar stimulation

(A) Multiple-site optical recording of the neural response in the sensory cortex induced by hypothenar stimulation. (B) Expanded traces labeled *a*-*d* in A. The pixel labeled *b* corresponds to the focus. (C) An isochrone map of the response onset constructed from data shown in A. The cross mark indicates the focus location. The interval of isochrone curves is 5 ms.

Figure 3 Example of optical recording of neural response

Multiple-site optical recording immediately after (A) and 30 min after (B) ulnar nerve injury. The PRE recording of this animal is shown in Fig. 2A. After ulnar nerve injury, neural responses to hypothenar stimulation were detected over the entire recording area. Pixels marked with a circle indicate the focus. Enlarged response signal at the focus of Ui (C) and Mi (D). The optical signals at the focus recorded before (dotted trace), immediately after (black trace), and 30 min after (gray trace) are aligned with the stimulus

timing and superimposed. The right panel in C or D shows the signal shown in the left panel normalized to the peak amplitude of each PRE signal.

Figure 4 Comparison of optical response signal at focus

Quantitative comparison of the peak amplitude (A), latency (B), and duration (C) of the response signal at the focus. In each figure, white, gray, and black bars indicate the data at PRE, at Ui0, Mi0, and Sa0, and at Ui30, Mi30, and Sa30, respectively. The length of the line on each bar indicates the standard deviation. In this and Fig. 6, individual data points are plotted as a gray circle and superimposed on bar graph. *: $p<0.05$.

Figure 5 Isochrone maps of evoked response for Ui (A) and Mi (B) groups

In each figure, left, middle, and right panels show the map at PRE, at Ui0 or Mi0, and at Ui30 or Mi30, respectively. The cross mark in each panel indicates the focus location. The interval of isochrone curves is 5 ms. The focus location varied among animals, probably due to differences in the relative position of the recording area and cortex. Since we did not compare the focus locations in the cortex among animals, little attention was paid to adjusting the relative position between real images and photodiode arrays.

Figure 6 Quantitative comparison of the propagation velocity of the excitatory wave among the directions of propagation

Four directions are compared: antero-medial (A), postero-medial (B), antero-lateral (C), and postero-lateral (D) directions (indicated as an arrow in each

inset). The meaning of the color of bar and the length of the line on each bar are the same as Fig. 4.

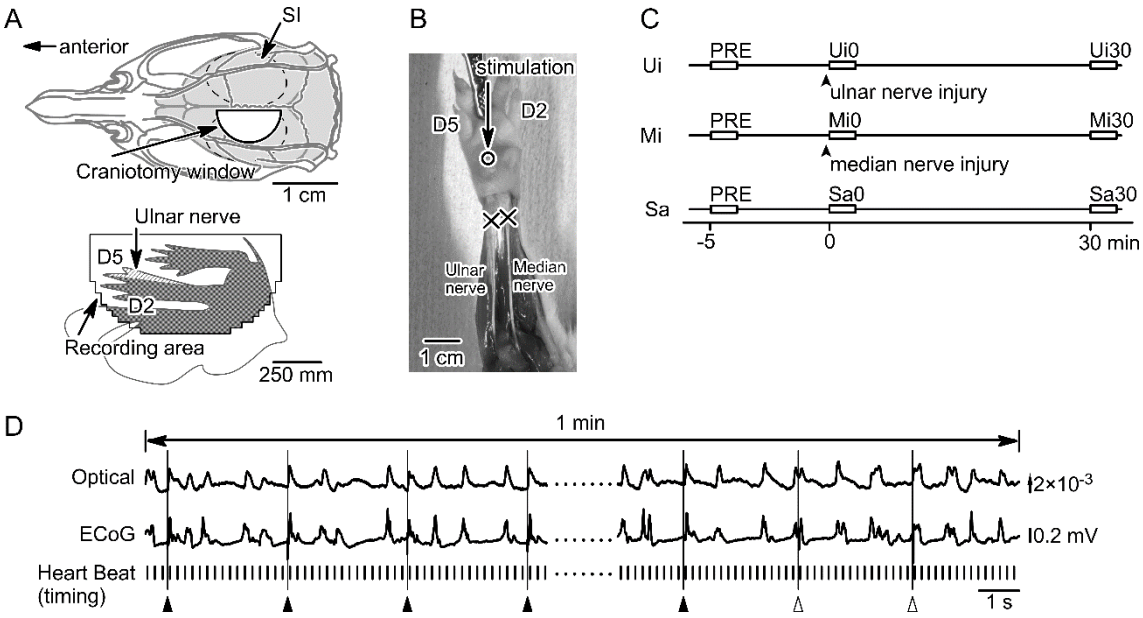
A
anterior
SI
Craniotomy window
1 cm
Ulnar nerve
D5
D2
Recording area
250 mm
B
stimulation
D5
D2
Ulnar nerve
Median nerve
1 cm
C
Ui
PRE
Ui0
Ui30
ulnar nerve injury
Mi
PRE
Mi0
Mi30
median nerve injury
Sa
PRE
Sa0
Sa30
-5
0
30 min
D
1 min
Optical
2×10-3
ECoG
0.2 mV
Heart Beat
(timing)
1 s

Figure 1

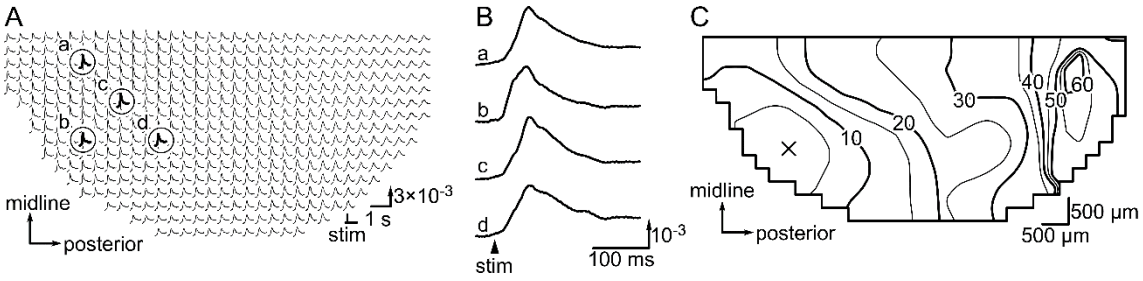
A
a
c
b
d
midline
posterior
3×10-3
1 s
stim
B
a
b
c
d
stim
10-3
100 ms
C
10
20
30
40
50
60
midline
posterior
500 µm
500 µm

Figure 2

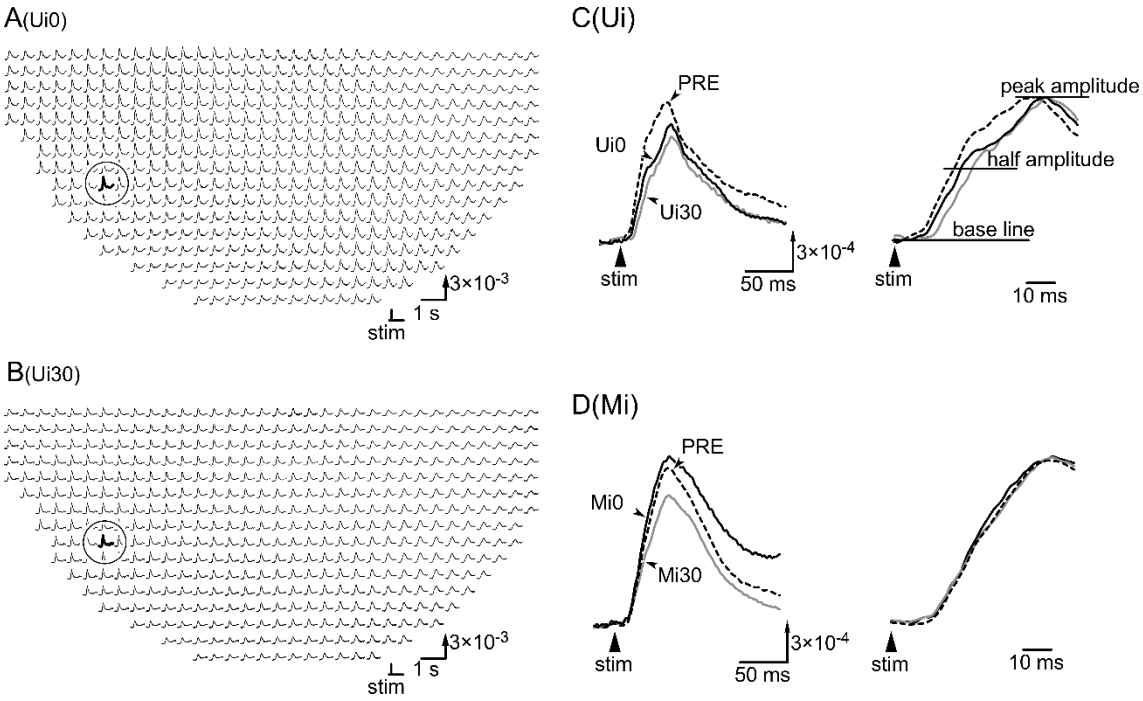
A(Ui0)
3×10-3
1 s
stim
B(Ui30)
3×10-3
1 s
stim
C(Ui)
PRE
Ui0
Ui30
3×10-4
stim
50 ms
peak amplitude
half amplitude
base line
stim
10 ms
D(Mi)
PRE
Mi0
Mi30
3×10-4
stim
50 ms
stim
10 ms

Figure 3

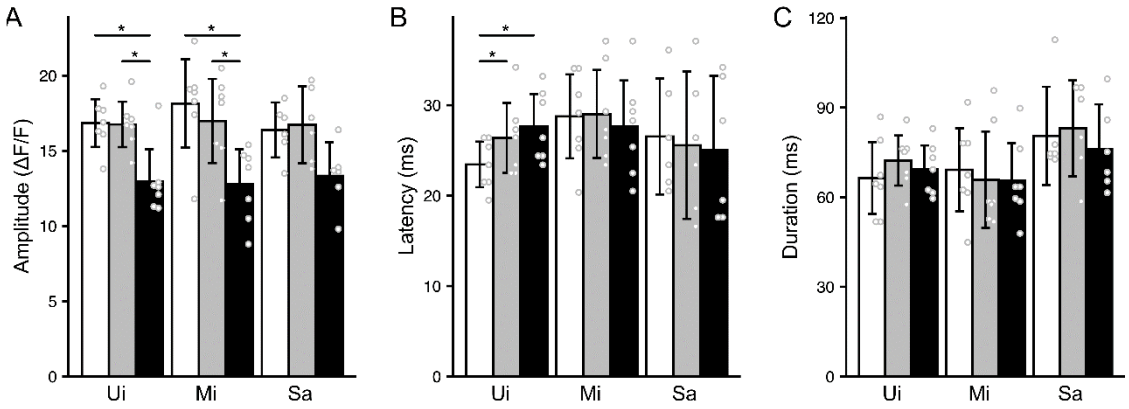

Figure 4

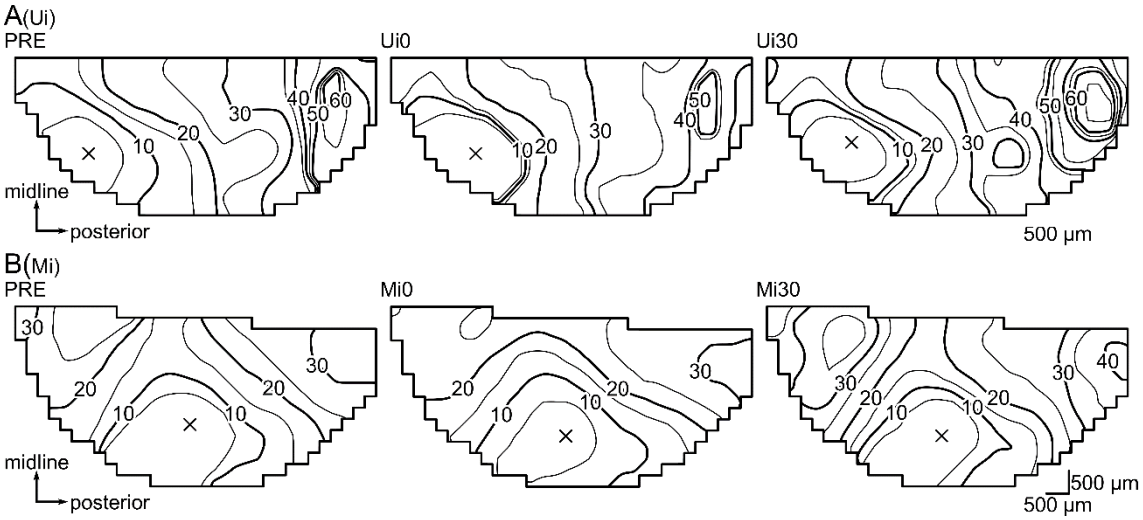
A(Ui)
PRE
Ui0
Ui30
midline
posterior
500 μm
B(Mi)
PRE
Mi0
Mi30
midline
posterior
500 μm
500 μm

Figure 5

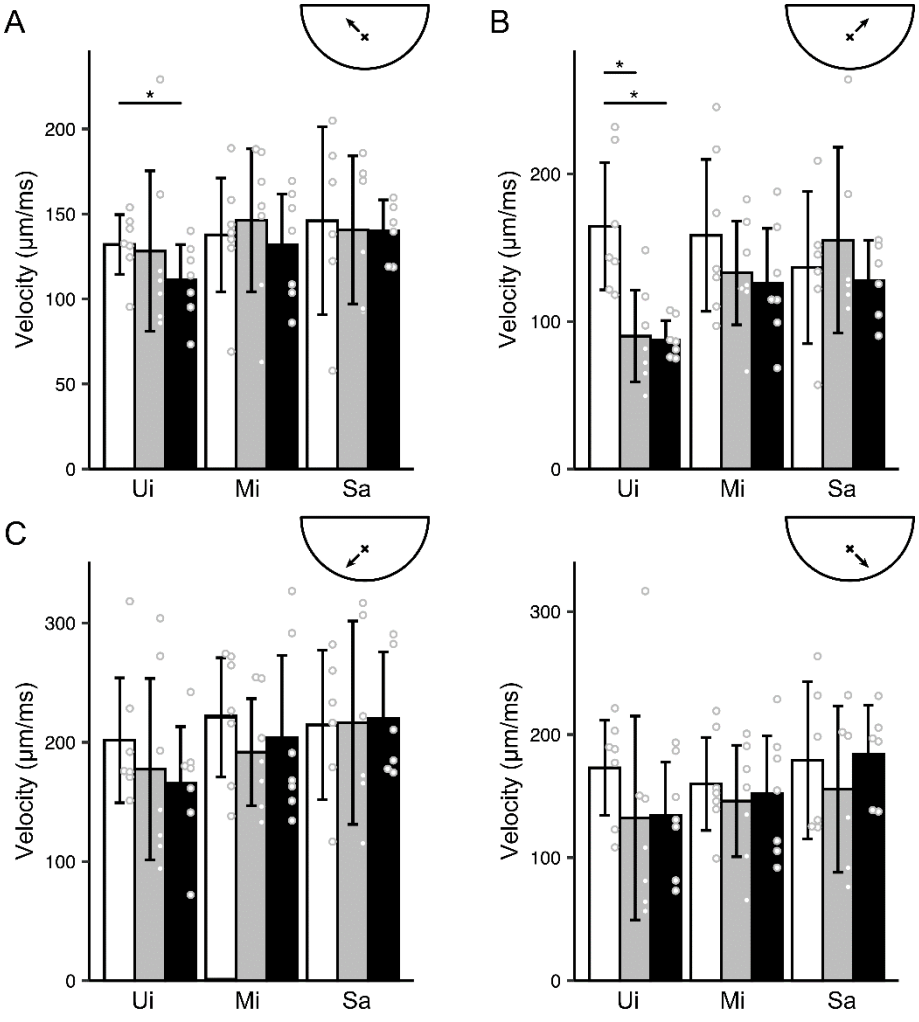

Figure 6