Neural circuits and temporal plasticity in hindlimb representation of rat primary somatosensory cortex: revisited by multi-electrode array on brain slices

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Abstract: Objective The well-established planar multi-electrode array recording technique was used to investigate neural circuits and temporal plasticity in the hindlimb representation of the rat primary somatosensory cortex (S1 area). **Methods** Freshly dissociated acute brain slices of rats were subject to constant perfusion with oxygenated artificial cerebrospinal fluid (95% O₂ and 5% CO₂), and were mounted on a Med64 probe (64 electrodes, 8×8 array) for simultaneous multi-site electrophysiological recordings. Current sources and sinks across all the 64 electrodes were transformed into two-dimensional current source density images by bilinear interpolation at each point of the 64 electrodes. **Results** The local intracortical connection, which is involved in mediation of downward information flow across layers II-VI, was identified by electrical stimulation (ES) at layers II-III. The thalamocortical connection, which is mainly involved in mediation of upward information flow across layers II-IV, was also characterized by ES at layer IV. The thalamocortical afferent projections were likely to make more synaptic contacts with S1 neurons than the intracortical connections did. Moreover, the S1 area was shown to be more easily activated and more intensively innervated by the thalamocortical afferent projections than by the intracortical connections. Finally, bursting conditioning stimulus (CS) applied within layer IV of the S1 area could successfully induce long-term potentiation (LTP) in 5 of the 6 slices (83.3%), while the same CS application at layers II-III induced no LTP in any of the 6 tested slices. **Conclusion** The rat hindlimb representation of S1 area is likely to have at least 2 patterns of neural circuits on brain slices: one is the intracortical circuit (ICC) formed by interlaminar connections from layers II-III, and the other is the thalamocortical circuit (TCC) mediated by afferent connections from layer IV. Besides, ICC of the S1 area is spatially limited, with less plasticity, while TCC is spatially extensive and exhibits a better plasticity in response to somatosensory afferent stimulation. The present data provide a useful experimental model for further studying microcircuit properties in S1 cortex at the network level *in vitro*.

Keywords: planar multi-electrode array; two-dimensional current source density imaging; primary somatosensory cortex; neural circuits; long-term potentiation

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1 Introduction

It has been widely accepted that neural circuits are the building blocks of complex neural networks in the central nervous system. Identities of information processing such

as representation, encoding and decoding, and computation of neural signals in terms of both spatial and temporal domains vary in response to dynamic exterior and interior environmental changes, leading to variation of neural circuits that perform different brain functions in different brain regions^[1]. Although neural circuits have been well studied in lower species of animal models, including the *Aplysia* siphon-gill withdrawal circuits, pyloric rhythm circuits of the lobster stomatogastric ganglion, and neural circuits of the locust antennal lobe-mushroom body system, the circuits in mammals remain still largely obscure, due to the structural complexity and the technical limitations^[1]. Nonetheless, studies in mammalian visual system have provided accumulative evidence indicating the existence of functional and structural neural circuits in the retina, the lateral geniculate body and the visual cortex^[1]. Moreover, the neural circuit in the hippocampal formation is closely linked to learning and memory functions of mammalian brain, thus having attracted special attention^[2]. Therefore, it is of particular importance to examine the properties of neural circuits in those higher brain structures, at the neural network level, to fully understand the sophisticated working principles and mechanisms underlying multiple brain functions such as perception, emotion, cognition and memory.

The primary somatosensory cortex (S1 area) is a well-known neocortical structure for processing various modalities of peripherally-incoming information (non-nociceptive and nociceptive), to produce sensory discrimination of touch, pressure, vibration, and pain, subserving the ability to analyze location, intensity and duration of the external stimulus^[3]. From the viewpoint of cytoarchitectonics and anatomy, the neocortical structure is featured by its laminar (layer) distribution parallel to the cortical surface^[3,4]. In addition, it has been proposed that the neocortex works in a unique columnar organization, namely the functional and structural unit, to represent peripheral receptive fields of the body^[3]. Specifically, the S1 area is mainly composed of 6 layers (laminae I-VI) with a mixture of different sizes of pyramidal and non-pyramidal cells^[3,5-8]. Studies to identify cell properties in layers II-VI within a column using single electrode recordings in anesthetized rats have revealed that non-noci-

ceptive neurons are primarily located across layers II-VI, while nociceptive cells are mostly located in layers V-VI, suggesting a layer-related difference in functional populations of neurons in S1 area^[9,10]. Moreover, similar results have been reported recently, by selective activation of peripheral A δ fibers using CO₂ laser stimulation to the rat tail^[4]. However, little has been reported concerning the layer-related difference in spatial and temporal characteristics of local neural circuits in the hindlimb representation of the rat S1 area either *in vivo* or *in vitro*, although the hind paw withdrawal reflex is mostly used as a quantitative index for nociceptive responses in both behavioral and electrophysiological recordings.

The planar multi-electrode array (pMEA) recording technique is a valuable tool for recording electrophysiological activities from multiple sites of various biological preparations over extended periods of time, at the neural network level. It can facilitate understanding of complex brain processes and functions in normal and pathological states^[11]. The non-invasive nature of this technique permits real-time analysis of long-term effects that are often difficult to be assessed by traditional electrophysiological techniques^[12-17]. To visualize the spatial and temporal information of pMEA recording more vividly, two-dimensional current source density (2D-CSD) imaging can be used to generate movies of neuronal activities within the neural circuit on a millisecond time scale resolution, by estimating the location and distribution of synaptic currents underlying recorded field potentials^[18-20]. The pMEA technique has already been successfully applied in many regions of brain slices^[11], such as the hippocampal formation^[18-22] and the anterior cingulate cortex^[23]. Since the structural layers of the S1 cortex are readily recognizable in living slices, physiological studies can be performed in an exact spatial frame *in vitro*^[24,25]. Recently, microcircuits of rat barrel cortex have been exquisitely examined by both multi-site tungsten electrode recordings and the MEA chip of 60 electrode arrays (Multichannel System, Germany)^[26,27]. The present study was aimed to investigate the spatiotemporal characteristics of neural circuits across different layers of the hindlimb representation of the rat S1 area, by a state-of-the-art Med64 system with 8×8 array,

coupled with the 2D-CSD imaging analysis method.

2 Materials and methods

2.1 Animals Male albino Sprague-Dawley rats (aged over 3 weeks, weighing 60–80 g) were provided by Laboratory Animal Facilities of both Capital Medical University (CCMU) and the Fourth Military Medical University (FMMU). Animals were housed (5 rats per cage) under standard laboratory conditions (12:12 h day/night cycle, at 22–26 °C under the humidity of 55–60%), with free access to food and water. All the experiments were approved by the Institutional Animal Care and Use Committee at both CCMU and FMMU. The number of animals used and their suffering were minimized as much as possible.

2.2 Slice preparation The general procedures for preparing acute S1 slices were similar to those for hippocampal and anterior cingulate cortex slices^[20,23]. Briefly, rats were decapitated after anesthesia with 4% sodium pentobarbital (40 mg/kg, i.p.), and the whole brain was quickly removed and placed into cold oxygenated (95% O₂ and 5% CO₂) artificial cerebrospinal fluid (ACSF) containing 124 mmol/L NaCl, 3.3 mmol/L KCl, 1.2 mmol/L KH₂PO₄, 2.4 mmol/L MgSO₄, 2.5 mmol/L CaCl₂, 26 mmol/L NaHCO₃ and 10 mmol/L glucose (pH 7.35–7.45). After 1–2 min, the brain block containing the hindlimb representation of the rat S1 area (from anterior surface of the optic chiasm to the posterior portion of the median eminence) was cut and trimmed (bregma 0.24 to –2.28). The stereotaxic coordinates were derived from the Rats Atlas of Paxinos and Watson^[28]. Then the 350- μ m slices were cut by a vibrating tissue slicer (Dosaka, DTK-1000). The transected slices were transferred to a chamber continuously perfused with oxygenated ACSF at room temperature. Slices were allowed to recover for at least 2 h before electrophysiological recording.

2.3 Preparation of the multi-electrode array A 64-channel multi-electrode dish system (MED64, Panasonic Alpha-Med Sciences, Japan) was used for extracellular field potential recordings in this study. Procedures for the preparation of the multi-electrode dish (MED) probe were almost the same as described previously^[20,23]. The size of each electrode in the array was 50 $\times$ 50 μ m², and all the 64 electrodes were arranged in an 8 $\times$ 8 square pattern with an inter-electrode distance of

300 μ m to cover a total area of 4.4 mm². Before use, the surface of MED64 probe was treated with 0.1% polyethyleneimine (Sigma, St. Louis, MO, USA) in 25 mmol/L borate buffer (pH 8.4) overnight at room temperature, followed by 3–5 times rinse with sterile distilled water. Generally, the MED64 probe could be re-used for approximately 30–40 recording sessions with a mean duration of 4–6 h.

2.4 Electrophysiological recordings After incubation, the slice was positioned on the MED64 probe, with the right S1 area being in the center of the recording dish that was mounted on the stage of an inverted microscope (IX71, Olympus, Japan). The anatomic locations of those recorded slices were equivalent to anterior-posterior (AP) 0.36–2.28 mm, medial-lateral (ML) 1.0–4.0 mm, and dorsal-ventral (DV) 0.5–4.5 mm, according to the Rat Atlas of Paxinos and Watson^[28]. Once the slice was settled, a fine mesh anchor (SHD-22L, Harvard, USA) was carefully disposed to ensure slice stabilization during recording. The slice was perfused with the same fresh ACSF at a rate of 2 mL/min with the aid of a peristaltic pump (PERI-STARTM, WPI, USA) through the whole period of electrophysiological recording.

After 20-min slice recovery, one of the 64 available planar microelectrodes was selected for stimulation after visual observation through a charge-coupled device camera (DP70, Olympus, Japan) connected to an inverted microscope (Fig. 1A). Non-specified, monopolar, biphasic constant current pulses (20–199 μ A, 0.1 ms) generated by the data acquisition software (Conductor 3.0, Panasonic Alpha-Med Sciences, Japan) were applied to layers II–III or layer IV of the S1 brain slice at 0.2 Hz. The evoked potentials were amplified by a 64-channel amplifier and then digitized at a 20 kHz sampling rate. The digitized data were displayed on the monitor screen and stored for offline analysis. To improve the signal-to-noise ratio, 5 successive responses were averaged automatically in real time by the recording system. The viability of the slices was kept constant across different sets of recordings by measuring the threshold for evoking field potential of adequate amplitude.

2.5 Experimental design After selection of sites in layers II–III for electrical stimulation (ES), an input-output (I–O) curve was first determined by recording the local field potentials in

response to a series of stimulations at ascending intensities, from 20 μA to 199 μA with a 20- μA stepwise increase. Due to the technical limitations of the stimulus generator, higher intensities ($> 199 \mu\text{A}$) were not applied in the present study. For the induction of long-term potentiation (LTP), the intensity of the test stimulus was first adjusted to elicit half of the maximum effect based on the I-O curve. Stable baseline responses were then monitored for at least 30 min before delivering the conditioning stimulus (CS) to layers II-III of the S1 area. To standardize tetanizing strength in different experiments, the CS intensity was the same as that of the test stimulation. The CS consisted of 6 burst sequences, with 10-s intervals. Each burst sequence consisted of 10 trains of burst with 4 electrical pulses delivered at 100 Hz (interburst interval: 100 ms). After CS, the test stimulus was repeatedly delivered (at the identical intensity as baseline) once every 10 min for more than 2 h, and the changes in LTP magnitude and duration were observed.

In another group of slices, almost the same procedures were conducted, except that the selected stimulation site was within layer IV.

2.6 Current source density analysis In the present study, the 2D-CSD was computed to identify current sources and sinks in any direction within the plane of each S1 slice. In general, the 2D current density I_m in the presence of a field potential Φ was given as follows:

$$I_m = - \left[\sigma_x \frac{\partial^2 \Phi}{\partial x^2} + \sigma_y \frac{\partial^2 \Phi}{\partial y^2} \right]$$

Since the measured field potential (Φ_{ij}) was recorded on a planar array of 64 electrodes, the second partial derivatives at the center of particular electrodes could be computed from the measured field potential on that electrode and its neighbors:

$$\frac{\partial^2 \Phi_{i,j}}{\partial x^2} \approx \frac{(\Phi_{i+1,j} - 2\Phi_{i,j} + \Phi_{i-1,j})}{\Delta x^2}, \quad \frac{\partial^2 \Phi_{i,j}}{\partial y^2} \approx \frac{(\Phi_{i,j+1} - 2\Phi_{i,j} + \Phi_{i,j-1})}{\Delta y^2}$$

By considering the medium as ohmic with homogeneous conductance ($\sigma_x = \sigma_y = \sigma$), and under the assumption of equidistant electrodes ($\Delta_x = \Delta_y = \Delta$), the normalized CSD (I_{ij}^*) could be defined and computed as follows:

$$I_{i,j}^* \equiv \frac{\Delta^2 I_{i,j}}{\sigma} \approx 4\Phi_{i,j} - (\Phi_{i+1,j} + \Phi_{i-1,j} + \Phi_{i,j+1} + \Phi_{i,j-1})$$

With the normalized CSD values at the center of the electrodes, it was possible to calculate the density at any point (x, y) within the 8×8 array using bilinear interpolation. The yellow color was used to represent the positive currents (sources), the blue color was used to represent the negative currents (sinks) and the black color was used to map the zero current. Finally, CSD images at selected time points were plotted across all the 64 recording sites for each group of slices.

2.7 Data analysis For quantification of the I-O relationship, the amplitude of field potentials was analyzed by the MED64 Conductor. For LTP data, the amplitude of evoked field potentials was normalized and expressed as the percentage to the averaged baseline value. The total number of effective field potentials ($> 20\%$ baseline) reliably recorded over the S1 area was counted by an experimenter unaware of the experimental design, and averaged across slices for each group. Only one slice per rat was subject to the Med64 recording (n indicated the number of slices). Data were presented as mean $\pm$ SEM. When necessary, the statistical significance was determined by unpaired Student's t -test. In all cases, $P < 0.05$ was considered as statistically significant.

3 Results

3.1 Electrophysiological identification of neural circuits in the hindlimb representation of the rat S1 area According to the previously reported functions and layer characteristics of the S1 area, 2 types of neural circuits were identified in brain slices *in vitro*. On one hand, the local intracortical circuit (ICC) was identified by ES through a single electrode at layers II-III where small- or medium-sized pyramidal and non-pyramidal cells are densely distributed. On the other hand, the thalamocortical circuit (TCC) was also characterized by ES at layer IV (granular cell layer) onto which the thalamocortical afferents predominantly terminate. Fig. 1A showed the raw recording data from the 8×8 array of the Med64 probe that overlapped with the microphotograph of one S1 slice. ES at layers II-III of the S1 area evoked a downward network response at layers III-V but with less output to layer I (blue

dot and traces in Fig. 1A). The field potentials were typically featured by a negative-going pre-synaptic volley [peak latency 3.0 ms; conduction velocity (CV) > 120 m/s] followed by a small delayed negative-going (peak latency: 8.3 ms) and large positive-going (peak latency: 16.4 ms) post-synaptic components (upper in Fig. 1B, position see blue * in Fig. 1A). However, ES at layer IV of the S1 area elicited a main upward network response within layers II-III and a small downward flow to layer V (red dot and traces in Fig. 1A). The field potentials were also typically featured by a negative-going pre-synaptic volley (peak latency: 4.8 ms; CV: 125 m/s) followed by a large delayed negative-going (peak latency: 9.8 ms) and positive-going (peak latency: 21.0 ms) post-synap-

tic components (lower in Fig. 1B, position see red * in Fig. 1A).

To see whether there is a layer-related difference in the connection size between ICC and TCC, the effective field potentials (>20% baseline) produced by a series of ES at ascending intensities were counted. The I-O functional curves showed that TCC exhibited a much larger size of synaptic connection than ICC, as reflected by the larger number of effective field potentials at each suprathreshold stimulation intensity when ES was applied at layer IV than at layers II-III (Fig. 2, left). This was further confirmed by the 2D-CSD imaging analysis (Fig. 2, right). TCC was revealed to make more synaptic contacts with S1 neurons in both mediolateral

Fig.1 A typical example of simultaneous multi-electrode array recordings using the MED64 probe (8×8, 64 electrodes) on one slice (left) containing the hindlimb representation of the rat S1 area. A: Representative network traces of local field potentials recorded across the remaining 63 sites, evoked by ES through one electrode within layers II-III (blue dot, for intracortical connections) or layer IV (red dot, for thalamocortical afferent connections) are superimposed on the original microphotograph of the brain slice positioned on the MED64 probe (single electrode size: 50 μ m; inter-electrode distance: 300 μ m). The numerical values indicate the number of electrodes on the MED64 probe from 1 to 64. B: A representative single trace of local field potential evoked by ES within layers II-III (#19, see light blue * in A) or at layer IV (#11, see red * in A). a-c: the peak latencies of the negative-going pre-synaptic volley (a) and early negative- (b) and slow positive-going (c) post-synaptic potentials elicited by ES within layers II-III (upper panel) or layer IV (lower panel).

Fig. 2 Comparison of the connection size between ICC and TCC by counting the effective number of local field potentials ($> 20\%$ baseline) across 8×8 array (64 electrodes). Left panel: stimulus-response curves of the averaged number of local field potentials evoked by ES at layers II-III (black symbols, for ICC, $n=6$ slices) or at layer IV (red symbols, for TCC, $n=6$ slices), at ascending intensities of 20-199 μA with a 20- μA stepwise increase. The sizes of both ICC and TCC were stimulus intensity-dependent within the ranges from 20 μA to 80 μA for ES at layers II-III and from 20 μA to 120 μA for ES at layer IV. The ceiling size of TCC was more widespread than ICC when ES intensity reached a plateau (100 μA for ICC, 140 μA for TCC). $^{**}P < 0.01$; $^{***}P < 0.001$. Right panel: The 2D-CSD images across the S1 area in response to ES at layer IV with intensities of 40 μA , 80 μA and 120 μA , respectively, indicating stimulus intensity-dependent increases in both current sinks (blue) and sources (yellow). Red dots indicate the sites of ES. Scale bar: 600 μm .

($> 1200 \mu\text{m}$ wide) and vertical (1200-1500 μm deep from the surface) ranges than ICC (mediolateral: $< 600 \mu\text{m}$ wide; vertical: 900-1200 μm deep). The time-varying 2D-CSD images over 8×8 array of the S1 area demonstrated the difference between the 2 circuits more clearly and vividly (Fig. 3). As shown in Fig. 3, local ES at layers II-III (red dots in Fig. 3A) immediately produced a current sink (blue color) within layers III-IV that may represent action potential propagation along the axons of excitatory cells (image at 4.0 ms). Afterward, post-synaptic potentials occurred within the same area with a mixture of early current sinks and late current sources (yellow color) (see images at 5.0 ms, 9.0 ms, and 16.0 ms). Besides, local ES at layer IV (red dots in Fig. 3B) resulted in a similar current sink-source profile, but with a higher current signal

intensity at each sweeping time point (Fig. 3B).

To shape the neural circuits induced by ES at the above 2 sites, schematic illustrations were drawn from the raw data ($n=6$ slices). As shown in Fig. 4, the information flow of ICC was mainly downward from layer II to layer VI (Fig. 4A), while the information flow of TCC was primarily upward, with small downward outflow (Fig. 4B). It was also apparent that the extension scope of TCC outweighed that of ICC. In addition, both types of network neural circuits were likely to form functional columns within the S1 area (Fig. 2-4), which is consistent with the previous reports^[9,10].

3.2 Comparison of I-O strength between ICC and TCC in the hindlimb representation of the rat S1 area To determine whether there is any difference in I-O strength between ICC

Fig. 3 Typical examples of time-varying 2D-CSD images of 8×8 array recordings across the hindlimb representation of the rat S1 area, captured from the time of ES (time zero) to 65 ms sweep time point (one image per 1 ms, totally 65 images). **A**: 2D-CSD images at sweep timing 4.0 ms (pre-synaptic volley), 5.0 ms, 9.0 ms and 16.0 ms (post-synaptic potentials) following ES at layers II-III (#12, see red dots, for ICC). **B**: 2D-CSD images at sweep timing 4.8 ms (pre-synaptic volley, 7.0 ms, 10.0 ms and 21.0 ms (post-synaptic potentials) following ES at layer IV (#28, see red dots, for TCC). The maximal intensity of 199 μ A was used for ES at both sites. The current signal intensity at each sweep time point within layer IV was higher than that at layers II-III. Blue color indicates current sinks, yellow color indicates current sources and black color indicates the baseline. Scale bar: 600 μ m.

Fig. 4 Schematic patterns of ICC and TCC within the S1 cortex. **A**: The information flow of ICC was likely to be mainly downward. ICC was restricted mainly within a column across layers I-IV. **B**: The information flow of TCC was mainly upward with less being downward. TCC was likely to be interconnected by thalamocortical afferent projections across neighboring columns along layers I-VI.

and TCC in the hindlimb representation of rat S1 area, the functional curves of the field potential amplitude were compared following delivery of a series of ES at ascending intensities (20-199 μA , with a stepwise increase of 20 μA) onto layers II-III or layer IV. As shown in Fig. 5A, the I-O curve of TCC was dissociated from that of ICC with the former shifting leftward to the latter, indicating a larger amount of information flow (input and output) within TCC. Moreover, the intensity of ES that exerted half of the maximal effect was 29.02 μA for TCC and 53.25 μA for ICC, respectively, suggesting much stronger synaptic responsiveness in TCC. Fig. 5B showed examples of field potential traces (#19 vs #12 electrode recording) evoked by layers II-III or layer IV stimulation. Notably, both the pre-synaptic volley and post-synaptic potential components demonstrated a marked increase with the ascending stimulation intensities, although the increase magnitude at layer IV was much larger than that at layers II-III (Fig. 5B).

3.3 Comparison of LTP induction between ICC and TCC in the hindlimb representation of the rat S1 area

To further evaluate the layer-associated difference in temporal synaptic plasticity of network responses evoked by stimulating ICC or TCC in the S1 area, a bursting CS was provided through the site of stimulation before the 2-h recording of LTP induction and expression. As illustrated in Fig. 6B, application of CS onto layer IV of the brain slice resulted in a long-lasting increase in the amplitude of late component of field potential that was maintained for over 2 h till the end of recording. However, for the same trace, the amplitude of the early pre-synaptic volley was not changed by the same CS (Fig. 6B, right). Among the 6 slices with CS at layer IV, the rate for successful LTP induction was 83% (5 out of 6 slices), 20% larger than pre-CS baseline (Fig. 6B, left). Nevertheless, when CS was applied within layers II-III, the amplitude of field potential was not significantly altered (Fig. 6A, right), indicating less change in synaptic strength and the failure in LTP induction (zero out of 6 slices tested) (Fig. 6A, left).

Fig. 5 Comparison of the strength of pre-synaptic input and post-synaptic responsiveness between ICC and TCC within S1 cortex, by measuring the amplitude of local field potentials (mV) in response to a series of ES at ascending intensities. **A:** Stimulus-response curves of the averaged amplitude of local field potentials evoked by ES at layers II-III (white symbols, for ICC, $n=6$ slices) or at layer IV (black symbols, for TCC, $n=6$ slices), at ascending intensities of 20-199 μA with a 20- μA stepwise increase. The increases in field potential amplitude of both ICC and TCC were stimulus intensity-dependent within the range of 20-80 μA for ES at layers II-III and within the range of 20-100 μA for ES at layer IV. The ceiling effect of TCC was larger than that of ICC when ES intensity reached a plateau (100 μA for ICC, 120 μA for TCC). *** $P < 0.001$. **B:** An example of stimulus intensity-dependent increase in amplitude by ES at layers II-III (#19, upper panel) or at layer IV (#12, lower panel).

Fig. 6 Comparison of LTP induction rate within ICC and TCC. Left figures show the 2-h recordings of network field potentials induced by ES at layers II-III (A, $n=6$ slices) or at layer IV (B, $n=6$ slices) with an intensity that induces half of the maximal field potential response (60–80 μA) following bursting CS at the same site with the same intensity. The amplitude of field potential was normalized to the baseline value. Time zero represents the start of CS. Bursting CS resulted in a 83% rate for successful LTP induction (5 out of 6 slices) within TCC, while the same CS failed to induce LTP in any of the 6 slices within ICC. Right figures are typical examples of local field potential pre-CS or 120 min after CS at layers II-III (A) and at layer IV (B).

4 Discussion

In the present study, we successfully established an experimental setup for the rat S1 slice recordings using a state-of-the-art pMEA (64 channels, 8×8 array) recording technique. Compared with the traditional electrophysiological recording using single electrode, the pMEA technique allows detection of neural network activities both spatially and temporally, and at multiple sites simultaneously^[11]. As shown in Fig. 1A, network field potentials could be instantaneously recorded across different layers of the S1 cortex when ES was applied through one electrode at a certain site. The advantages and disadvantages of the pMEA technique application in brain slices *in vitro* have been discussed in detail in our previous reports^[20,23].

The major findings of the present study include: (1) description of network response patterns for intracortical connections and thalamocortical afferent connections on the same acute S1 brain slice, with a high signal-to-noise ratio (Fig. 1A), which can facilitate further direct comparisons of physiological and pharmacological properties in the brain matrix; (2) identification of 2 typical spatial neural circuits (ICC and TCC) within the S1 cortex across layers I–VI, both of which are likely to work in accord with the columnar principle that is frequently reported in the visual cortex, the barrel cortex of the S1 and the frontal lobe of the animals^[3,29–33]; (3) transformation of the analog electrophysiological signals into current sink and source images using 2D-CSD analysis method, allowing direct display of time-varying lapse of current signals within ICC and TCC in a frame of 8×8 array; (4)

analysis of different electrophysiological properties between ICC and TCC in terms of network synaptic connection size, information flow, I-O strength, and temporal plasticity.

4.1 Spatial map of neural circuit in the hindlimb representation of the rat S1 area The S1 cortex has a typical cytoarchitecture as other neocortex and consists of 6 parallel layers^[3]. Layer I (molecular layer) contains horizontally running axons and dendrites, small nonspiny and nonpyramidal cells, and Cajal-Retzius cells. Layer II (corpuscular layer) contains nonpyramidal cells such as small basket cells. Layer III (pyramidal layer) contains medium-sized pyramidal cells and nonspiny, nonpyramidal cells (the largest basket cells, the chandelier cells and double bouquet cells). Layer IV (granular layer) contains many small neurons, and cell bodies are tightly packed in a narrow layer (including the spiny, nonpyramidal cells, neurogliform cells, *etc.*). Layer V (ganglionic layer) contains large pyramidal cells. Layer VI (pleomorphic cells) contains cells of varying morphology (Martinotti cells, *etc.*). Since layers III, V and VI have corticofugal projections to contralateral cortical area, subcortical nuclei and spinal cord, respectively, only layers II and IV are mostly involved in intracortical connections. Particularly, layer IV mainly receives the thalamocortical afferent projections. Thus, layers II-III and layer IV were selected as stimulation sites for spatial mapping of local neural circuit within the S1 area. Consequently, 2 typical local microcircuits are identified. One is ICC that is likely to link the inter-layer connections across layers I-IV, and the other is TCC that is likely to link the thalamocortical afferent terminals to the local neuronal cell bodies and dendrites by synaptic connections across the 6 layers. Here we find that the information flow activated by the thalamocortical afferents spreads upward to layers II-III, with less input to deep layers V-VI (Fig. 4B), while the information flow activated by local activation within layers II-III spreads downward to layer VI from layer II (Fig. 4A). This result is consistent with the information flow map recorded in the living slices of rat barrel cortex^[26,27]. Regarding the difference in allocation of the thalamocortical information flow, it is interesting to compare the cell population mapped by *in vivo* electrophysiological studies in the hindlimb representation of the rat S1 area. Previous studies

have found that 29% of the neurons, mainly located within layers II-VI, could be activated by non-nociceptive inputs, while 13% of the cells, mainly located within layers V-VI, could be activated by nociceptive inputs or convergent inputs^[9,10]. Besides, 58% of the cells without definite peripheral receptive field are localized in an intermingled manner across the whole 6 layers. Based upon the previous reports, we propose that the major information flow conveyed by non-nociceptive and nociceptive afferents is likely to project to both superficial and deep layers of the S1 area via a common path of the microcircuit TCC. For the microcircuit ICC, the major information flow serves as an intracortical modulatory network that can be tonically activated by continuous thalamocortical input or inter-cortical input regardless of the specific input properties. However, it should be noted that the local circuit is likely to be re-organized or changed by peripheral persistent nociception under pathological conditions. In our previous immunohistochemical study, we observed a robust increase in activation of c-Fos-expressing cells within layers II-III of the hindlimb representation of the rat S1 area following contralateral injection of bee venom into the hindpaw pad, a model of persistent inflammatory pain^[34]. Future experiments are directed towards explicating the effect of peripheral persistent nociception on the spatial map of local circuitry in the S1 cortex.

The layer IV in S1 cortex contains dense cellular aggregates, and it is proposed that layer IV spiny neurons function as strong amplifiers of even weak thalamic inputs since they are highly interconnected^[35]. This morphology of the network map may boost the stream of excitatory synaptic activity from the thalamus, causing widespread activation at the end^[33,35]. Most previous *in vivo* and *in vitro* studies have found that in rat barrel cortex, thalamic stimulation leads to strong initial excitation of layer IV, inducing a series of excitation through the cortical column, first reaching supragranular and then infragranular layers^[35,36]. In contrast, layer III pyramidal cells that have been thought to represent the major cell type are likely to be tonically regulated by inhibitory interneurons *per se*^[37]. As a consequence of this strong tonic inhibition, it seems less likely that the intrinsic neural circuitry linking around layers II-III will be susceptible to any

external influence. As a piece of supporting evidence, in the present study, ES application at layer IV produced a much larger synaptic connection and field potential amplitude than application at layers II-III, suggesting a better plasticity of TCC than that of ICC (Fig. 2, 3 and 5).

Taken together, within the hindlimb representation of the rat S1 area, there are refined spatial maps of synaptic connection and transmission that might represent the activation of different local neural circuits with layer-related differences in network response pattern, connection size, orientation of information flow, individual synaptic output efficacy and temporal plasticity (see below) under physiological state.

4.2 Temporal plasticity of neural circuit in the hindlimb representation of the rat S1 area In the current study, the waveforms of network field potentials within the 2 types of neural circuits are relatively consistent. Typically, after ES through one electrode at the site of stimulation (SOS), a compound potential was evoked around the SOS, consisting of a sequence of immediate negative-going component followed by slow negative- and positive-going components, regardless of the direction of information flow within the microcircuits of either ICC or TCC (Fig. 1B). It has been demonstrated that the early negativity is a pre-synaptic volley, while the late components belong to postsynaptic potentials^[38]. Moreover, we also observed field potentials around the SOS that only possess slow negative- and positive-going postsynaptic components without a pre-synaptic volley. Based upon the conduction velocity of each component, we propose that the slow potentials should be a result of excitation of apical dendrites of layer V pyramidal cells or axons of layer IV spiny cells that extend far from the activation site of synaptic contacts. This is supported by a previous finding that synaptic contacts onto the layer IV cells are mostly confined to the dendritic arbor near their mother cell body^[35].

It is interesting to find that there is a significant difference in temporal plasticity of neural circuit between ICC and TCC. LTP, an activity-dependent enhancement of synaptic strength, is thought to be important for the adaptation of behavioral functions, as well as higher functions such as learning and memory^[2,39]. Consistent with a previous report,

we show that LTP can be induced in the neocortex in a similar way to that in the hippocampus^[40]. However, LTP induced in the neocortex is proven to be related with CS parameter. For example, the optimal form of CS to induce LTP in rat S1 cortex slice is suggested to be high frequency stimulation (HFS), rather than low frequency or theta burst stimulation^[38]. Moreover, induction of LTP is also associated with the region of neocortex for application. For instance, LTP can be easily induced in granular cortex such as S1 area, but not in the agranular primary motor cortex^[41]. Here as an input, application of CS onto layer IV of the S1 brain slice resulted in a high rate (83.3%) of successful LTP induction, while application of the same CS within layers II-III failed to induce LTP (Fig. 6). This suggests that the plasticity of TCC is better than that of ICC, due to the different functional properties of the 2 circuits. That is, TCC serves as a functional module processing sensory information from the periphery to produce sensory discrimination, while ICC is involved in maintaining the morphology of the functional column. However, whether the spatial and temporal properties of the local neural circuit in the S1 cortex would be changed under pathological pain states still remains to be further examined.

4.3 Significant implications The hindlimb representation of the rat S1 area is a very important brain structure involved in the processing of somatosensory input of both nociceptive and non-nociceptive signals from the contralateral hindlimb. Nociceptive reflex and pain-related behaviors are often measured on the hindlimbs. However, little is known about the structure and function of the S1 cortex representing the hindlimbs. In the present study, we successfully identified the spatially functional map of 2 patterns of neural circuits in the hindlimb representation of rat S1 area, which may provide a useful experimental model for further investigation of nociceptive information processing *in vitro* at the network level. Furthermore, we have also found a layer-related circuitry-difference in spatially synaptic connection and temporally synaptic potentiation in the S1 cortex, which may help further investigate the cortical mechanisms underlying abnormal changes in sensory discrimination of stimulus modalities (such as pain, hyperalgesia, allodynia and paresthesia) under various pathological states.

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大鼠初级躯体感觉皮层后肢代表区的神经回路和时间可塑性：平面微电极阵列记录技术的应用

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摘要: **目的** 运用平面微电极阵列记录技术探讨大鼠初级躯体感觉皮层(primary somatosensory cortex, S1 area)后肢代表区的神经回路和时间可塑性。**方法** 将急性分离的大鼠脑片置于MED-64系统的电极平皿中, 持续灌流通入95% O₂和5% CO₂混合气的人工脑脊液, 随后进行多电极同时同步记录。利用双线性内插法计算出64个点的电流源与电流井, 并将其转换为二维电流源密度分布图。**结果** 刺激S1后肢代表区脑片的II-III层, 可鉴定出局部皮层内的连接回路, 而刺激IV层(丘脑传入的主要终末端)则鉴定出丘脑皮层间的连接回路。首先, 丘脑皮层投射的激活可以诱发更多的有效场电位, 并且与S1区更多的神经元建立突触联系。与之相比, 皮层内回路的激活引起的电反应活动范围则较小。其次, 刺激IV层在每一刺激强度下诱发的场电位幅度远远大于刺激II-III的场电位幅度, 即S1区更容易被丘脑皮层连接回路的投射纤维所激活。最后, 在S1区IV层给予强直刺激可以记录到长达两个小时的局部场电位幅度的增加, 长时程增强(long-term potentiation, LTP)的诱出率约为83.3% (5/6例)。相反, 在II-III层, 同样的强直刺激并未能明显诱导LTP的发生。**结论** 大鼠S1区的后肢代表区似乎存在两种不同类型的神经回路, 一种是由层间联系介导的皮层内回路, 另一种则是由丘脑皮层传入介导的丘脑皮层连接回路。皮层内回路在空间上联系较局限且可塑性不强, 而丘脑皮层回路的空间连接范围则相对较广, 对躯体感觉信息的传入也显示出很强的可塑性。本结果为进一步在体外神经网络水平研究S1区的神经回路特性提供了很好的实验模型。

关键词: 平面微电极阵列记录技术; 二维电流源密度分析; 初级躯体感觉皮层; 神经回路; 长时程增强