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Temporal strategy for discriminating noxious from non-noxious electrical stimuli by cortical and thalamic neural ensembles in rats

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Abstract

Considerable evidence supports that pain is encoded in a large, widespread network that consists of the thalamus, cortex, as well as limbic system. However, the temporal properties of the neural matrix in pain processing were largely unknown. In the present study, we simultaneously recorded thalamic and cortical neuronal discharges elicited by brief noxious or innocuous electrical stimulus in awake rats. The discrimination performance of the neural ensembles in differentiating noxious from innocuous inputs was calculated using different window sizes at the millisecond and second level, respectively. The results demonstrated that coding information emerged in a quantum-like manner; the minimum spike-train length for discriminating noxious from innocuous inputs was 40 ms. The nociceptive coding activity was temporally dynamic, and could be preserved for a relatively long time (3–4 s) within the thalamocortical loops, independent of the initial brief stimuli. These results suggest that the nociceptive signals may be reverberatory within the thalamocortical loops, hence keeping the neurosignature for central pain representation.

Keywords: Cortex; Electrical stimulation; Neural ensemble; Pain processing; Spike train

Many areas of the brain are involved in the experience of pain. Numerous studies have shown that pain is encoded in a large, widespread network that consists of the thalamus, cortex, as well as limbic system [5,21]. The concept of "pain network" or "pain neuromatrix" has been well established. Moreover, pain is considered to be represented by some spatial and temporal neural activity patterns (the neurosignature) [15]. The neurons within pain network has been demonstrated to have high complex temporal dynamics during nociceptive processing [3,4,15]. However, our understanding of the temporal properties of the supraspinal structures in pain processing was still limited. It is known that the central nervous system depends on discrete action potentials or spikes to encode somatosensory information, including pain [18]. The temporal sequence of spikes and the duration of spike trains provide important information about how the brain works when we feel pain [6]. An inference from the neuromatrix theory leads to the hypothesis that some special patterns of spike activities, which are differed from those evoked by somatosensory inputs within a similar distributed network, will be the optimal central representation of pain (i.e., the pain signals) [16]. Although we can easily realize how long a pain sensation lasts, we have little knowledge about how long a pain signal persists in the brain during either acute or chronic pain states. In general, the existence of acute pain always parallels with a stimulus or an injury. For example, sustained noxious stimuli can produce continuous sensation of pain throughout the stimulus [2]. Although acute pain evoked by brief noxious inputs has been extensively investigated, and their sensory transmission mechanisms are believed to be well understood, less well defined is the temporal pattern with which the stimuli are represented in the neuronal population responses. Previous studies have described that a sufficient time-span is needed for the encoding of an external stimulus in the brain. For instance, the natural calling songs can be distinguished perfectly using a 100-ms spike train by grasshopper auditory system [14]. Thus, it is of interest to investigate how much time is required for the brain to discriminate noxious from non-noxious stimuli, thereby forming a perception of pain out of a wealth of somatosensory inputs. Revealing the time length of ensemble spike-train activities required for pain perception as well as the temporal

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distribution of these spike trains after a brief noxious stimulus will provide insight into how brain represents pain and picks up nociceptive signals from numerous somatosensory inputs. It will also help to verify the theory of neuromatrix and neurosignature.

In the present study, we simultaneously recorded the thalamic and cortical neuronal discharges elicited by a brief noxious or non-noxious electrical stimulus in awake rats. The time function of the neural ensembles in differentiating noxious from innocuous inputs was examined using a variable time window. By calculating the information carried by neuronal populations at both millisecond and second level, i.e., the capability of neural ensembles to discriminate pain from non-pain signals, we attempt to reveal the temporal coding strategy of central neural spike trains for the perception of pain. Our hypothesis is that if the neuromatrix theory is true, quantum-like ensemble coding blobs with unique temporal patterns will be observed instead of a continuous or random distribution of coding capacity.

Six adult male Sprague-Dawley rats weighing 250–300 g were used in this study. All subjects were individually housed under a 12-h dark–light cycle (lights off from 7:00 to 19:00) and allowed free access to water and food. The experimenter gently handled the animals every day to make them get used to the manipulation.

On the surgery day, rats were anesthetized with intraperitoneal injections of sodium pentobarbital (Nembutal, 50 mg/kg) and mounted on a stereotaxic apparatus. Following the retraction of skin and soft tissue, four small craniotomies were made over the recording sites using stereotaxic coordinates. Arrays of eight stainless steel Teflon-coated microwires (50 μm diameter, Biographics, Inc., USA) were lowered into the unilateral primary somatosensory cortex (SI) (1.0 mm posterior (P) to bregma, 2.0 mm lateral (L) to midline, and 2.0 mm ventral (V) from the skull surface), the anterior cingulated cortex (ACC) (−3.2 P, 0.8 L, 2.8 V), the parafascicular nucleus of thalamus (Pf) (4.2 P, 1.3 L, 6.0 V), and the ventroposterior lateral nucleus of thalamus (VP) (3.0 P, 3.2 L, 6.0 V). The microarrays were cemented to the animal's skull with dental acrylic using skull screws as anchors. The animal care and experimental procedures were also approved by the Chinese Academy of Sciences and followed the guidelines of International association for the study of Pain (IASP).

All experiments were conducted in awake rats following a recovery period of 5–7 days. Animals were slightly restricted in a hanging-up waistcoat with their heads, limbs, and tails moving freely. Rats were trained to get familiar with the waistcoat for 3–4 times till they became habituated to it. Electrical stimuli were generated by a stimulator Pulsemaster A-300 and DC powered isolator A365 (World Precision Instrument, Inc., USA). Single rectangular pulses were delivered to the glabrous skin of the hindpaw contralateral to the microwire implant via a pair of platinum electrode pads. Conductive paste was smeared between electrode pads and skin.

In the experimental session, noxious stimulations (6-mA current, 2-ms duration) were delivered, which were randomly intermixed by innocuous (control) pulses (0.5 mA, 2 ms) at an interval of no less than 25 s. There were around 160 trials for each animal, a half for pain and another half for control. Additional 10–15 noxious stimulations were delivered prior to the recording session and the data were not included in the final analysis. The nociceptive responses were compared with non-nociceptive ones for all the recording units to explore the specific central nociceptive coding pattern. The intensity of 6 mA used here was determined as noxious because stimuli over 5 mA can reliably evoke pain in the conscious investigator according to Sikes and Vogt [19]. Villanueva et al. used similar stimulation parameter (0.66 Hz, 2-ms duration) and reported that the mean threshold for C-fibers activation was 2.7 ± 0.5 mA [22].

The simultaneous extracellular activity of all single units was recorded throughout the duration of the stimulation experiment. Neural electric signals were obtained from the stainless steel microwires and passed from the headset assemblies to a preamplifier via two lightweight cables and a commutator. The time resolution for data collection was 50 kHz. The signals were band-pass filtered between 0.5 and 5 kHz (6 dB cutoff) before being sent to a spike-sorting device. Spike activities were monitored on a computer. Waveforms were picked up by setting proper parameter pairs for amplitude and duration, and recorded into a database file with a PC-based software *Magnet* (Biographics, Inc., USA). The identity of clearly sorted single neurons was verified by graphical capture of waveforms. Data were then analyzed with commercially available PC-based programs *STRANGER* (Biographics, Inc., USA) and *Nex* (Plexon, Inc., USA). A time stamp series (resolution, 1 ms) marking the electric stimuli presentation was recorded and synchronized with the neuronal spike recordings.

At the termination of the experiment, the rats were deeply anesthetized with sodium pentobarbital. Recording sites were marked by electrophoretically deposited iron (20 μA, 10–20 s) at the tips of selected wires. Animals were then perfused with 4% paraformaldehyde and their brains were extracted. The brains were post-fixed in a solution of 5% potassium ferricyanide/4% paraformaldehyde for several days. Coronal sections (40 μm) were cut through the SI, ACC, and thalamus. The iron deposits were easily identified as blue dots. Data obtained from the microwires outside the target regions were not included in the analysis.

The average firing rate was quantified for each neuron using peri-event time histograms (PSTHs). The bin size was 100 or 5 ms for the computation of PSTHs with a time range of −3 to 5 s or −100 to 300 ms, respectively. Bin counts were calculated in *Nex* and exported to *MatLab*, where neuronal firing rates were transferred into Z scores: $Z = (X - M)/S$, where Z is the normalized value of firing rate (Z scores), X is the actual firing rate obtained from PSTH, M and S are mean and standard deviation of the basal neuronal discharging, respectively. Z scores were arranged in clusters to visualize the response pattern in neuronal populations. A sliding-window method was used to examine the difference between nociceptive and non-nociceptive neuronal responses with Student's t-test, as well as the difference in the percentage of responsive neurons between noxious and innocuous stimuli with Chi-square test. The difference was considered significant only when it reached a significance level of P-value < 0.005 in three consecutive windows, thus to achieve

a global significance of $P < 0.05$. The P-values produced by the sliding-window method were converted into *Surprise* ($-\ln P$) for the purpose of highlighting the significance [1].

A linear discrimination method was selected in the analysis. Discriminant function analysis is used to determine which variables discriminate between two or more groups (in this case, noxious and innocuous stimulations). The basic idea underlying discriminant function analysis is to determine whether groups differ with regard to the mean of a variable, and then to use that variable to predict group membership (e.g., of new cases). This multivariate statistical method has been used extensively for neural ensemble data analysis [7,17]. Here we used it to search for the different patterns of ensemble neural activity associated with different sensory stimulations, thus to estimate the capacity of distinguishing painful from non-painful events for a neuronal population within a given brain area.

Briefly, all the neuronal responses within a given area were used to calculate the principal components (PCs) with software from the *Nex* program. Then the PCs were exported to *MatLab* with 50-ms bin size for the larger-scale data (−3 to 5 s poststimulus) and 2-ms bin size for the smaller-scale data (−100 to 300 ms poststimulus). A sliding window, in which the PCs were averaged across the window time length, was moving along the time axis at 1-bin step. Then a matrix consisting of PCs × trials was obtained and allowed to perform discriminant analysis. Computation with different window sizes was employed. By increasing the window size stepwise (0.2–2 s low-resolution windows for the larger-scale analysis and 20–200 ms high for the smaller), we can explore the contribution of temporal coding to the neural ensemble performance.

A muscle twitch in the hind limb could be observed when the electrical stimulation was delivered. Animals did not vocalize during the application of weak (0.5 mA) electrical stimuli, while intense electrical stimuli (over 5 mA) evoked vocalization in them. No obvious struggle activity was observed throughout the experimental session.

A total of 123 units (45 from ACC, 12 Pf, 40 SI, and 26 VP) were recorded. The intense electrical stimuli (6 mA) evoked nociceptive neuronal responses characterized by stronger magnitude, longer duration, and involved larger number of neurons in contrast to the non-nociceptive response evoked by 0.5 mA stimulation (Fig. 1A).

The neural ensemble discrimination performance was examined using different sizes of moving windows. Initially, neuronal spike activity of a larger time scale (3 s before and 5 s after stimulation) was analyzed. Fig. 2A showed an example of the ACC neural ensemble performance to distinguish the two types of stimuli. Interestingly, the ability to discriminate the two stimuli changed with both the window size and the window position, indicating that most of the information-bearing spike trains may emerge within certain time-range after stimulation. Thus, perfect performance can be obtained only with certain window size and at some particular poststimulus time. For example, the time window that contained the maximum discriminant information was found at the position of 1.2 s poststimulus and with the window length of 1.3 s (i.e., the spike train of 0.55–1.85 s post stimulus, see Fig. 2A). In addition, the ensemble discrimination performance was temporally dynamic over the poststimulus time (Fig. 2B), suggesting that the nociceptive encoding activity was discrete. This individual result was corroborated by the average analysis across all subjects. As shown in Fig. 2C, the discriminant activity of ACC, Pf, SI and VP ensembles peaked twice or more during poststimulus time (3–4 s) period. Signals from missing wires failed to display similar phenomenon (data not shown). This suggests that the nociceptive information could be preserved for a relatively long time within the thalamocortical loops, independent of the initial brief stimuli. Whether this information might possibly contain mixed affective response of fear to the stimulus will be determined in later studies with chronic pain models [10].

In an attempt to identify the spike-train length for the discrimination, we scanned a smaller time scale at millisecond level (−100 to 400 ms) with higher-resolution windows (20–200 ms). We found that there were many focused 'discrimination blobs', where the nociceptive information can be perfectly differentiated from the non-nociceptive (Fig. 3A). It is noteworthy that

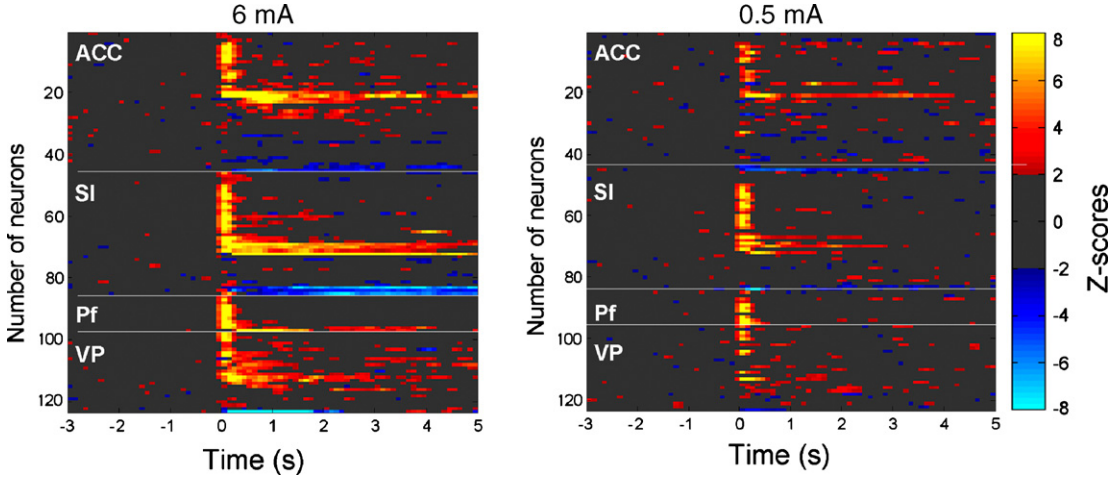

Fig. 1. Clustered neuronal responses induced by noxious and innocuous electrical stimulation. The firing rate is normalized to the baseline level (warm color for the excitatory response and cold for the inhibitory). In contrast to the non-nociceptive responses, the nociceptive ones were characterized by stronger magnitude and/or longer duration in some of the neurons, and larger number of neurons involved.

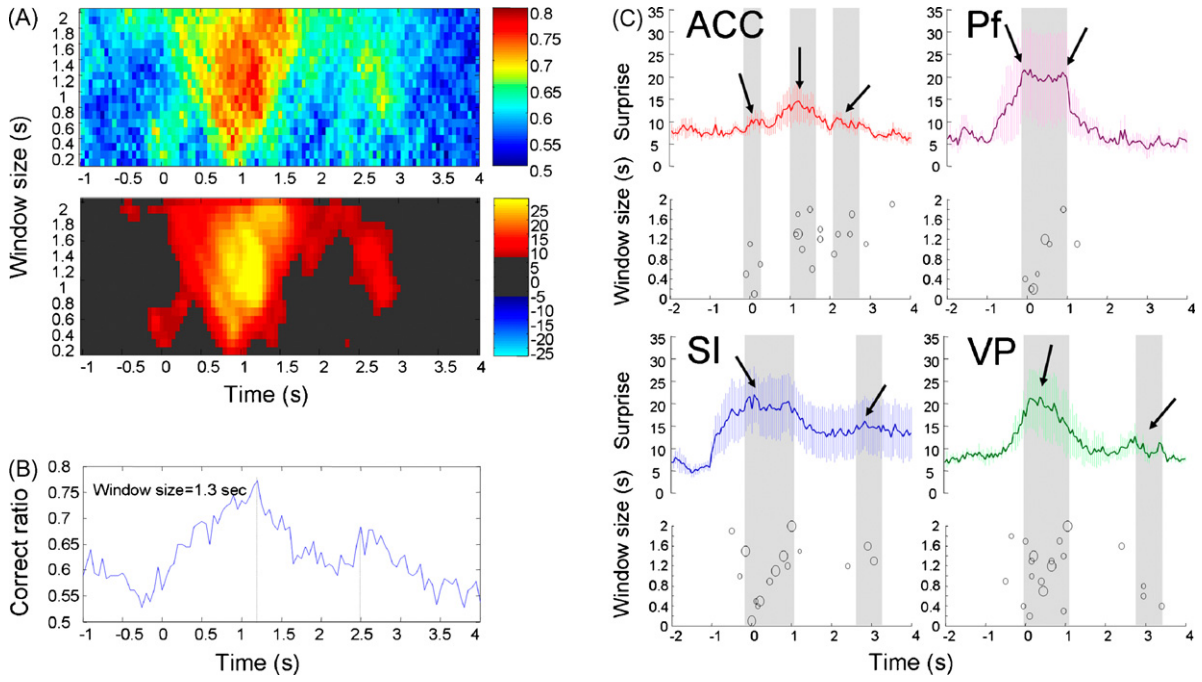

Fig. 2. The neural ensemble discrimination performance computed with different window sizes on a larger time scale (2 s before and 4 s after stimulation). (A) An example of ACC ensemble performance to distinguish noxious from innocuous stimuli. The colored plots, as in the following figures, represent the percent of correct discrimination between noxious and innocuous stimuli (top panel), and the significance (expressed as $-\ln P$, generated by comparison between correct and chance performance) (bottom panel), respectively. As shown here, the performance increases with the window length. The perfect performance occurs at the particular window size (1.3 s) and poststimulus time (1.2 s). (B) An example showing that ensemble discrimination performance was temporally dynamic over the poststimulus time. As can be seen here, the discrimination performance peaked twice at poststimulus 1.2 and 2.5 s when the window length is fixed at 1.3 s. (C) The summarization of the discrimination performance of ACC, Pf, SI and VP ensembles across all the subjects and over the window sizes. The top and bottom rows of each subplot represent averaged and scatter-plotted performance, respectively. The size of the circles corresponds to the discrimination ability of the spike trains at the sampled window size (as in the following figure). As indicated by arrows and shaded areas, the discrimination activity peaked twice or more for all the simultaneously recorded areas.

when the window length was shorter than 40 ms, no discriminant effects could be detected (Fig. 3A). The distribution of 'discrimination centers' across subjects was scatter-plotted in Fig. 3B. The averaged window sizes for good discrimination performance were 123.0 ± 7.2 ms (ACC), 131.7 ± 7.1 ms (SI), 132.0 ± 10.5 ms (Pf), and 138.0 ± 7.2 ms (VP), respectively. Given that the size of the circles corresponds to the discrimination ability of the spike trains, we can infer that Pf and SI neurons have the best ability to discriminate nociceptive information from non nociceptive information; that of VP is better than ACC but worse than Pf and SI. It is also reasonable to conclude that the longer spike-trains yielding perfect performance observed on the larger time scale may be composed of many such smaller discriminant centers.

The present study investigated the temporal coding pattern of the pain networks for the brief noxious electrical stimulation. The results have demonstrated that discrete 'discrimination blobs' (ensemble spike train of certain length that bears information) distributed as a function of time after stimulation; the minimum spike-train length for discriminating noxious from innocuous inputs is 40 ms. The nociceptive coding activity is temporally dynamic, and could be preserved for a relatively long time (3–4 s) within the thalamocortical loops, independent of the initial brief stimuli.

Numerous evidence support the idea that the perception of pain due to acute injury or chronic pain states undergoes substantial processing at supraspinal levels, including the thalamus, cortex, as well as limbic system. In the present study, we simultaneously recorded many single-neuron activities within the thalamocortical pain network. We found that the nociceptive and non-nociceptive information transmitted by the neuronal activity looked quite different, if viewed by a 100-ms bin and with a 3-bin *Gaussian* smooth (see Fig. 1A). Which part of these differences is sufficient for the brain to distinguish pain from normal sensory inputs? Our results demonstrate that most of the information pops up in the first 150 ms after stimulation, especially in the thalamus and the primary somatosensory cortex. In the natural world, discrimination of natural stimuli is of great importance for many animals. For example, male grasshoppers have to detect and discriminate calling songs in different length from potential mates [14]. Rats use whisker information to identify tactile input and to determine the shape and orientation of an obstacle [11,20]. As far as pain is concerned, feeling of pain elicited by sharp points or burning heat could make one promptly escape and avoid further injury. Thus, it is obvious that distinguishing pain from miscellaneous tactile inputs at very early stage has great physiological significance.

We also found that spike-train length longer than 40 ms was necessary to discriminate between noxious and sensory stimuli. The average ensemble spike-train length for discrimination turned out to be around 130 ms (including both thalamic and cortical neurons). There are two possible explanations for this

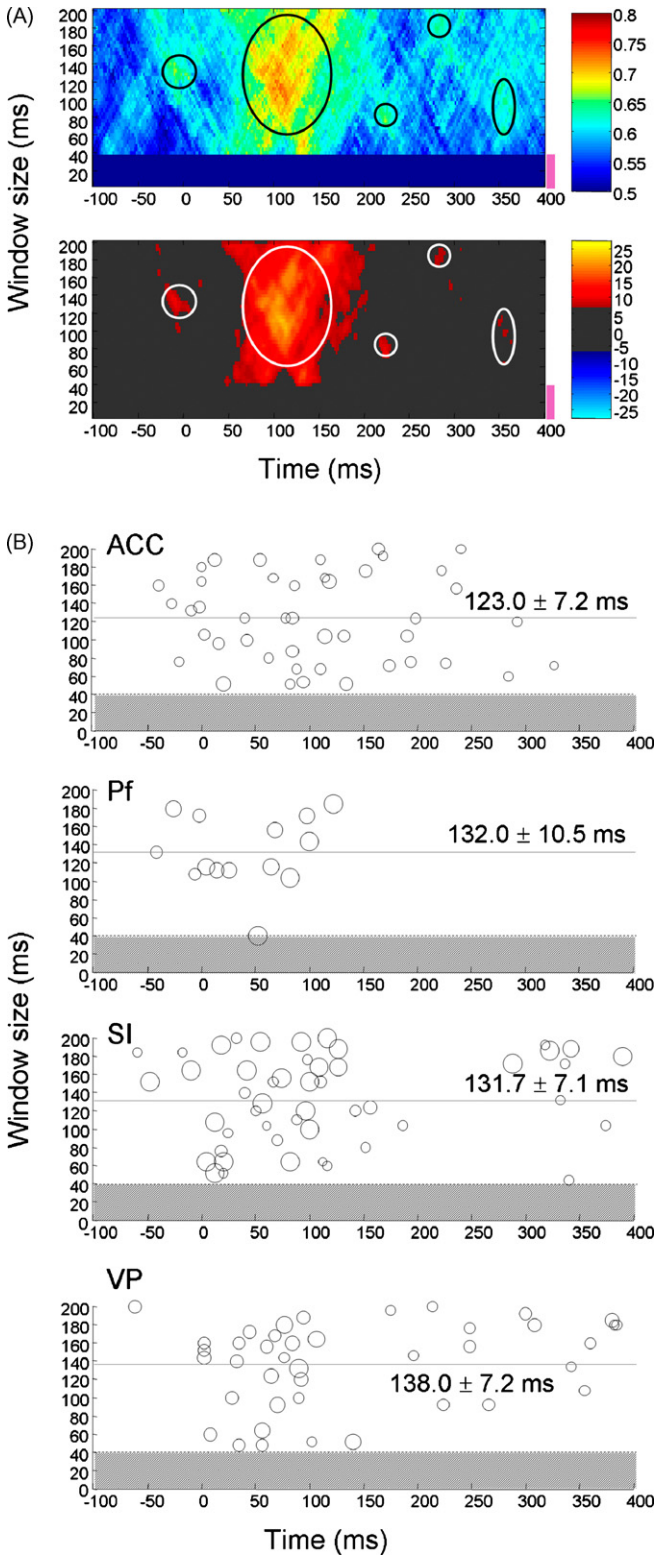

Fig. 3. The 'discrimination centers' calculated at millisecond level (−100 to 400 ms) with higher-resolution windows (20–200 ms) using the same data as Fig. 2A. (A) The positions of the 'discrimination blobs' (circles) indicate where the nociceptive information can be perfectly differentiated from the non-nociceptive. Notice that when the time window is narrower than 40 ms, no discriminant effects can be detected). (B) Scatter plots of the 'discrimination blobs' across all animals. The averaged window sizes for good discrimination performance were 123.0 ± 7.2 ms (ACC), 131.7 ± 7.1 ms (SI), 132.0 ± 10.5 ms (Pf), and 138.0 ± 7.2 ms (VP), respectively.

phenomenon. First, this time length is believed to permit the development of a conscious sensation [9]. Libet et al. found in humans that if the skin of the hand was stimulated with a suprathreshold stimulus, the sensation could be prevented by stimulating the cortex hundreds of milliseconds *after* the skin stimulus [12]. This means that to elicit a conscious sensation requires substantial cerebral durations. Accordingly, we infer that in the case of painful stimulation, the time period that the neural ensembles needed for the discrimination should be a temporal strategy for the brain network to achieve a conscious pain experience. Second, considering the random nature of our multiple neuron sampling, this phenomenon may reflect the temporal dynamics with which the discriminating information reverberates in the neural networks, as described by Lau and Bi [11].

Additional finding of our study was that the meaningful discriminant performance existed for up to 3–4 s following a brief electrical stimulus. When a brief stimulus applied on the periphery resulted in prolonged activation of higher neural centers, two conditions should be considered. First, the nociceptive signals from the periphery produced sustained activity in the dorsal horn neurons and drove the upstream neurons discharge strongly. Second, the nociceptive inputs were reverberatively transmitted in the thalamocortical circuits and produced abundant synchronized discharges among neurons. It seemed that the former do not fit the present results, because the peripheral receptors and dorsal horn neurons were less likely to be persistently activated by a brief electric pulse. Only tissue or nerve injury that leads to the release of chemical mediators can produce sustained discharges in the nociceptors. Livingston once postulated that there was a reverberatory circuit in the dorsal horn where constant nociceptive signals from the periphery generate prolonged activity in the dorsal horn neurons, which then transmit abnormally patterned volleys of nerve impulses to the brain [13]. This postulation was considered an explanation for the persistent pain. In contrast, Melzack proposed a neuromatrix theory in which the nociceptive inputs from the body undergo cyclical processing and synthesis in the distributed neural network which can persist even after the injury has been cured [15]. Our previous results also showed that the sensory information flowed bidirectionally (bottom-up and top-down) in the thalamo-cortical neural circuits after a cutaneous electrical or chemical stimulation [8,23]. Therefore, the present findings may be associated with more feedforward and feedback mechanisms at higher brain levels. Based on the above theories and our previous results, it is possible that the perception of acute pain involves the sustained activation of supraspinal brain networks.

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