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Pain

Supraspinal electrophysiological models for studying chronic pain

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Electrophysiology has been intensively used in studying molecular mechanisms of pain transmission, modulation and plasticity. *In vitro* slice recordings provided detailed information on synaptic transmission and plasticity under both physiological and pathological pain conditions. *In vivo* recordings integrate the information of functional activities in the nociceptive pathway. Combination of electrophysiology with genetically manipulated mice, new behavioral models, *in vivo*/*in vitro* imaging and molecular biology will identify new molecular targets for controlling chronic pain in the future.

Introduction

Pain is an unpleasant experience of sensation induced by a noxious stimulus. Physiological pain is important for animals to avoid potential injury, whereas pathological pain like chronic pain is annoying, lasts for an extended period of time after injury and is characterized by a heightened responsiveness to both noxious and non-noxious stimuli (named as hyperalgesia and allodynia, respectively). Chronic pain costs $100 billion annually in healthcare and lost productivity. Our knowledge of the pain pathway and transmission has significantly improved over the last decades; however, the cellular mechanisms of chronic pain remain to be elucidated. More rational treatment of chronic pain is based on the understanding of the pathophysiological mechanisms underlying the various symptoms and characteristics of chronic pain. It is noted that the treatment of chronic pain is challenging because the ability to feel acute pain is essential for mammals and humans to survive. The new generation of drugs will need to selectively reduce chronic pain without interfering with acute pain [1].

It is now generally believed that chronic pain involves both peripheral and central components of the nervous system, including the pain-related cortex. Chronic pain is likely to share some common cellular mechanisms with memory, considering the strikingly similarity between synaptic plasticity in spinal cord and hippocampus [2]. However, we proposed that chronic pain is not only the memory of pain, but also is akin to a 'mega-memory' event. The synaptic plasticity occurs in both spinal cord and supraspinal structures in pain pathways. In addition, the brain is likely to receive continuous abnormal neuronal activity from the injured areas as long as the inflammation or injury persists. Therefore, brain changes are apt to be the summation of plasticity on top of plasticity. Recent results have shown that pathological pain caused dramatic changes in the brain, from receptors and ion channels, basal synaptic transmissions, to activity-dependent plasticity. Targeting central plasticity is becoming the direction for finding pain relieving medications [3].

Owing to the complex etiology of chronic pain, in which many plastic changes take place at the levels of proteins and genes, it is not sufficient to employ classic pharmacological and behavioral approaches to investigate such mechanisms.

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Electrophysiology is a powerful tool to investigate functions of the nervous system. Field recordings, single unit recordings, intracellular recordings and patch clamp recordings have been successfully applied both *in vitro* and *in vivo* to probe the functional changes after chronic pain. Utilizing a combination of electrophysiology with molecular biology, imaging, different pain models and genetically manipulated animals have greatly facilitated our understanding of cellular mechanisms on chronic pain. In this review, we first present an overview of the electrophysiological approaches in studying chronic pain. We then summarize recent progress arising from the use of *in vitro* and *in vivo* electrophysiological approaches, with particular emphasis on patch clamp electrophysiology in studying chronic pain at the supraspinal level.

Electrophysiological approaches: functionally dissect the cellular mechanism of pain

When typing in 'electrophysiology and chronic pain' in PubMed, we will get more than 500 papers related to the topic. Electrophysiology has been widely used in pain studies to determine functional changes underlying the development of chronic pain conditions. Chronic pain is associated with prolonged tissue damage or injury, which results in complex changes in the nociceptive pathway, from dorsal root ganglion (DRG), spinal dorsal horn, brain stem, thalamus and finally to pain-related cortex as somatosensory cortex, anterior cingulate cortex (ACC) and insular cortex. These changes include alterations of receptor and ion channels, neurotransmitters or even neural structures, leading to the change in the neuronal network in the pathway and the subsequent behavioral sensitization [3].

Electrophysiology is the study of measuring electric activity of neurons, involving voltage change or electrical current flows, which reflect the function of neurons and the neuronal network. Extracellular recordings as single unit recordings and field potential recordings allow us to record action potential activities and massive potential changes in tissue, whereas intracellular recordings and patch clamp recordings are used for the measurement of voltage or current in a single neuron or single channel. By using these electrophysiological approaches, we can detect the expression of electrical signals, the main functional output of neuronal network in the nervous system. Considering the functional alterations in the nociceptive pathway under pathological conditions, electrophysiology is definitely an effective approach to dissect its cellular mechanisms. For example, synaptic transmission, modulation and plasticity have been studied in DRG neurons where painful stimuli are transduced, in the dorsal horn where the first central synaptic relay for pain signals occurs and in supraspinal structures such as the ACC, a region of the brain important for the negative emotional components of pain [4].

Patch clamp electrophysiological studies have provided detailed insights into the molecular mechanisms underlying nociceptive processing. Combined with *in vitro* spinal slice preparations and cultured DRG neurons, patch clamp studies have addressed basic synaptic transmission and plasticity as well as functional changes after chronic pain from DRG to spinal cord [5,6]. In addition, long-term synaptic plasticity as long-term potentiation (LTP) and long-term depression (LTD) of synaptic responses have been shown to occur in dorsal horn neurons both *in vitro* and *in vivo* [7,8]. Most recently, it has been reported that inflammatory pain also induced the LTP of responses in dorsal horn *in vivo*, suggesting LTP is the synaptic amplifier of chronic pain at the spinal level [9]. With whole-cell recording, recent studies found that long-term disinhibition in the spinal cord can contribute to persistent pain caused by nerve injury [10]. All these studies suggest that patch clamp electrophysiology provides a powerful tool to study the functional mechanism of chronic pain at spinal level.

In vitro electrophysiological studies of chronic pain

Nociceptive transmission starts from DRG, then to spinal cord dorsal horn and finally convey to supraspinal structures such as brain stem, thalamus and pain-related cortex. At each relay, sensory synapses are under precise regulation to provide appropriate behavioral responses. *In vitro* electrophysiological approaches have been applied to almost all of these nuclei under both physiological and pathological conditions. Among these, DRG and spinal dorsal horn received particular attentions, and electrophysiological studies in these nuclei are well characterized [6]. Here we will mainly focus on electrophysiological studies of the supraspinal structures after chronic pain. Although chronic pain is likely due to long-term plastic changes along the nociceptive pathway from the periphery to the cortex, we will review synaptic transmission and plasticity in supraspinal structures using *in vitro* electrophysiological approaches. The plasticity in the supraspinal structures probably represents late long-term changes after injury, which might be more relevant to chronic pain observed in patients.

Anterior cingulate cortex

Recent studies from both human and animal tests consistently suggest that the ACC and its related areas are important for processing pain perception. ACC neurons respond to nociceptive stimuli and activity within the ACC is related to the unpleasantness or discomfort of somatosensory stimuli [11]. Electric stimulation or chemical activation of ACC induced pain and fear in animals [12,13], whereas blocking excitatory transmission or downstream cAMP and AC1&8 pathway inhibited behavioral sensitization [14]. In addition, peripheral injury caused bilateral activity in the ACC, for example, the increased immediate early gene expression,

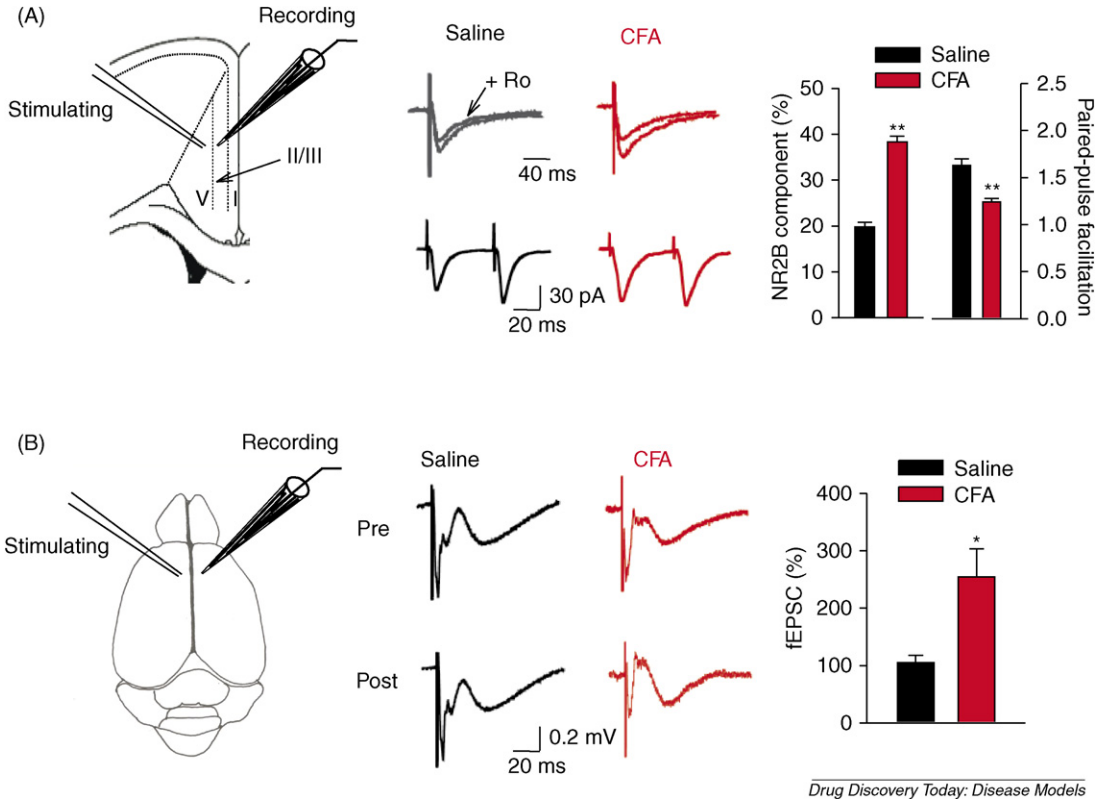

Figure 1. *In vitro* and *in vivo* electrophysiology showing plastic changes in the anterior cingulate cortex after peripheral CFA inflammation. (A) Whole-cell patch clamp recordings in the slice of anterior cingulate cortex. Evoked responses were obtained in neurons from Layer II-III by electric stimulation in the Layer V. NMDA responses are recorded in the presence of $GABA_A$ receptor antagonist, picrotoxin (100 μM) and AMPA receptor antagonist, CNQX (20 μM) (upper traces). NR2B component is revealed by perfusion of selective NR2B antagonist, Ro256981 (3 μM) (shown by the arrow). NR2B component is significantly increased after CFA inflammation. The paired-pulse facilitation (PPF, 50 ms interval) of AMPA receptor-mediated EPSCs is recorded in the presence of picrotoxin and AP5 (50 μM), a NMDA receptor antagonist (lower traces). PPF is significantly decreased in the inflamed mice, suggesting enhanced presynaptic glutamate release after inflammation. ** $P < 0.01$. (B) *In vivo* field recordings in the anterior cingulate cortex. Evoked field excitatory postsynaptic potentials (fEPSPs) were obtained by electric stimulation in the contralateral cingulate cortex. Significant increase of NMDA receptor-mediated fEPSPs is found after CFA inflammation (post) but not from saline group. * $P < 0.05$.

such as c-fos, Egr1 and CREB, and increased electrophysiological responses [15-17].

Electrophysiological experiments in cortical slices have shown that excitatory synaptic transmission in the cingulate cortex is primarily glutamatergic [18,19]. With whole-cell patch clamp and field recordings, both LTP and LTD of excitatory transmission can be induced in ACC slices [17,20–22], which allow a more detailed examination of the mechanisms underlying cortical plasticity. Both NR2A and NR2B NMDA receptors are required for LTP induction in the ACC. The downstream multiple signaling pathways such as calmodulin, calcium-stimulated AC1 and AC8, CaMKIV, MAPK are involved in LTP in the ACC [21–24].

Recent studies demonstrated that central plasticity occurs in the ACC after chronic pain. Genetic forebrain overexpression of NR2B increased the NMDA-mediated postsynaptic current in the ACC and enhanced the behavioral sensitization after inflammation [15]. Most recently, we found that peripheral inflammation caused the upregulation of NR2B in the ACC, which may underlie the inflammation-related persistent pain [18]. With whole-cell patch clamp recordings, postsynaptic NMDA receptor-mediated excitatory synaptic current is recorded. Our results showed that NR2B NMDA receptor-mediated component is increased in mice with complete Freund's adjuvant (CFA) inflammation (Fig. 1A). However, the neuronal excitability itself in the ACC is not altered after inflammation [18]. Moreover, our recent results further showed that presynaptic release of glutamate in the ACC is increased after peripheral inflammation [25]. After detailed analysis of electrophysiological data on pair-pulse facilitation, miniature excitatory postsynaptic currents (EPSCs) and MK801 blockade on NMDA receptor EPSC, we consistently found the increased release probability in chronic pain mice (Fig. 1A) [25]. In addition, our results showed that the LTP in the ACC was decreased in the mice with CFA inflammation [25]. Considering the important role of NR2B in the induction of LTP [22] and upregulation of NR2B in the ACC after inflammation [18], the loss of LTP is surprising. The reasonable explanation is that LTP may be occluded by the enhanced presynaptic glutamate release [25].

Using amputation model and field recordings, we found that amputation of the third hindpaw digit in an adult rat caused a loss of LTD that persisted for at least 2 weeks [17]. LTD might help to maintain appropriate neuronal activity within the ACC by reducing synaptic transmission. Our results suggest that synaptic LTD in the ACC may also contribute to enhance neuronal responses to subsequent somatosensory stimuli after amputation [16].

Insular cortex

Human imaging and clinical studies have well document that the insular cortex is involved in pain, particularly related to emotional part of pain perception [26]. However, *in vitro* electrophysiological studies in this brain area are rare. Using field recording, it has been reported that NMDA receptor-mediated response are important for pain responses. For example, our studies showed that forebrain NR2B overexpression increased NMDA receptor EPSCs in the insular cortex and behavior sensitization [15]. A report using PSD-93 knockout mice showed that persistent pain induced by tissue inflammation or nerve injury was significantly reduced in the knockout mice, in part because of the decreased NMDA receptor-mediated responses in the insular cortex [27]. Consistently, a recent study reported that NMDA receptor transmission contributes to network hyperexcitability in rat insular cortex using intracellular recordings [28].

Long-term plasticity in the insular cortex is not well characterized. Using field recordings, we found that theta-burst stimulation induced LTP in insular slices [24]. The LTP is diminished in CaMKIV knockout mice, suggesting the involvement of the CaMKIV pathway in insular LTP [24]. Although we believe that long-term plasticity in the insular cortex is important for chronic pain, there is still lack of any evidence to support this notion for the time being. However, as discussed below, some studies did show that *in vivo* LTP in the insular cortex is involved in the conditioned taste aversion [29,30]. Future *in vitro* electrophysiological studies are needed to address cellular mechanism of insular plasticity and its role in chronic pain.

Somatosensory cortex

Somatosensory cortex is important for determining the location and quality of noxious stimulation. It is well known that somatosensory cortex under reorganization of cortical representational maps after peripheral denervation such as amputation [31]. However, plastic changes after chronic pain in somatosensory cortex do not seem as dramatic as in the ACC, suggesting its comparatively minor role in pathological pain. For example, there is no upregulation of NR2B-mediated EPSC or increased presynaptic glutamate release in the somatosensory cortex after peripheral inflammation [18,25]. In addition, there is no loss of LTD in the somatosensory cortex after amputation [17].

Using *in vitro* slice recordings, we have shown that the somatosensory cortex can exhibit LTP induced by theta-burst stimulation [24] and LTD induced by low frequency stimulation [17]. However, it has been reported that there is a critical developmental period for induction of LTP or LTD in the somatosensory cortex [32], which may partially explain the less plastic changes in pathological pain. It is interesting to note that thalamocortical synapses can be formed as silent synapses, which are subsequently made functional by LTP induction [32].

Thalamus

The thalamus plays an essential role in processing and relaying nociceptive information to the pain-related cortex. It has been reported that stimulation in the human somatosensory thalamus can reproduce both the affective and sensory dimensions of previously experienced pain [33]. In addition, the thalamus may play an important role in the pathological pain, because plastic changes also happen in the thalamus after chronic pain. For instance, long-term rearrangement reorganization within the thalamus occurs after peripheral nerve injury [34]. However, unlike the ACC, there is no upregulation of NR2B NMDA receptor-mediated responses in the ventrobasal thalamus after peripheral CFA inflammation [18].

It is important to study synaptic transmission and plasticity in the thalamus; however, very few studies exist. Recent studies using dual whole-cell recording found that activation of metabotropic glutamate receptors causes long-term reduction of electrical synapse strength between the inhibitory neurons of the rat thalamic reticular nucleus [35]. Moreover, whole-cell recordings revealed that there are intrathalamic interactions between dorsal thalamic nuclei [36]. However, the role of the modulations in chronic pain is currently unknown.

Midbrain and brain stem

Nuclei as midbrain periaqueductal gray (PAG), rostral ventral medulla (RVM) and nucleus raphe magnus (NRM) in brain stem are important relay stations in the nociceptive pathway. In particular, these nuclei are critical in endogenous descending inhibition or facilitation [37]. The RVM relays descending influences from the PAG and ACC to the spinal cord to form endogenous analgesia systems (PAG-RVM-spinal cord) and facilitatory system (ACC-RVM-spinal cord) [37]. Despite their critical roles in the nociception, less is known about synaptic transmission and plasticity in these nuclei.

Chronic pain caused functional changes in all these nuclei. A recent study showed that BDNF in the PAG and BDNF receptor TrKB in the RVM are upregulated after inflammation, which may contribute to the endogenous descending modulation [38]. In the brain stem, it has been reported that both AMPA and NMDA receptors undergo selective

transcriptional and translational modulation in RVM pain modulatory circuitry following inflammation [39]. Electrophysiological studies found that chronic morphine treatment increase both GABAergic and glutamatergic transmission in the NRM [40].

In vivo electrophysiological studies of chronic pain

In vitro electrophysiology in slice preparation provides a useful tool for studying the cellular mechanism of chronic pain at different levels. However, the disadvantage of *in vitro* electrophysiology is that slice preparation is only two-dimensional; both descending and ascending projections are eliminated. Moreover, physiological stimulations such as touch, pinch and other sensory modalities cannot be used. To address these technical limitations, *in vivo* electrophysiology was clearly needed.

In vivo electrophysiological approaches such as single unit recordings and field recordings in anesthetized animals and freely moving animals are well characterized to study pain transmission and modulation. These *in vivo* electrophysiological approaches have been applied to most nuclei involved in the nociceptive pathway under both physiological and pathological conditions. More recently, *in vivo* patch clamp recordings in anesthetized or even in freely moving rats were also developed. Using *in vivo* patch clamp recording, Yoshimura group successfully studied the process of sensory transmission in the spinal cord and sensory cortex [41]. A recent study reported a novel method using *in vivo* whole-cell recordings in freely moving rats. With a miniature head-mountable recording device, the recording pipette rigidly stay put after the whole-cell configuration is established. Neurons in both motor cortex and hippocampus were successfully recorded [42]. We expect these novel *in vivo* electrophysiological approaches will soon be used in studying cellular mechanisms of chronic pain.

Pain-related cortex

Consistent with *in vitro* electrophysiological studies in ACC slices, *in vivo* recordings in anesthetized or freely moving rats showed that ACC undergo changes under both physiological and pathological conditions. Field responses recorded in the ACC could be obtained by electric stimulation of hindpaw, anterior thalamic nuclei or within ACC in anesthetized rats [16,43]. The responses showed both short-term and long-term plasticities [16,43]. After digit amputation, there is a long-term enhancement of field responses in the ACC [16]. Consistently, similar increase of a long-lasting membrane potential depolarization was also obtained by using intracellular recordings of single neuron in the ACC *in vivo* after digit amputation [18]. The amputation-induced LTP in ACC neurons might be associated with the synaptic mechanisms for phantom pain. Using freely moving mice, we also tested the field excitatory postsynaptic potentials (fEPSP) in the ACC after peripheral CFA inflammation. We found that NMDA-receptor mediated fEPSPs are significantly enhanced 1 day and 3 days after inflammation, which may be due to the upregulation of NR2B in the ACC (Fig. 1B) [18].

In vivo whole-cell patch clamp recordings have been applied to the somatosensory cortex in anesthetized rats. Oscillatory activities having 0.5–5 Hz frequency and up to 500 pA amplitude were recorded in cortical neurons. The tough stimuli to a single whisker elicited a large amplitude barrage of EPSCs [43]. *In vivo* studies in the insular cortex are mainly focused on the basolateral amygdala to insular cortex pathway, which are known to be important for the aversive taste learning. Results showed that the LTP is NMDA-receptor dependent and involves the activation of extracellular regulated kinase pathway [29,30]. However, the potential changes in these cortical areas after chronic pain are not well studied with *in vivo* electrophysiology.

Thalamus and brain stem

Most *in vivo* electrophysiological studies in thalamus and brain stem are from single unit recordings. All these nuclei reported enhanced activities after chronic pain. However, the detailed molecular mechanisms of the modulation are not well characterized due to the technical limitation of single-unit recordings. Results have shown that thalamic neurons exhibited enhanced firing rates after chronic spinal cord injury [44]. RVM neurons also can undergo plastic changes after tissue injury or inflammation [45]. There is close interaction between RVM and PAG under chronic pain conditions. For example, injection of formalin modifies RVM neuronal activities and this effect is prevented by PAG cannabinoid receptor stimulation, which requires the mGluR5 activation in the PAG [46]. The plasticity and hypersensitivity in these nuclei are likely to be part of the central reorganization producing behavioral sensitization after chronic pain.

Conclusions

The use of electrophysiology has provided great insight into the molecular mechanisms of pain transmission, modulation and plasticity. *In vitro* slice recordings, mainly whole-cell patch clamp recordings, provided detailed information on synaptic transmission and plasticity under both physiological and pathological pain conditions. *In vivo* recordings in anesthetized animals or freely moving animals integrate the information of functional activities in the nociceptive pathway. Therefore, electrophysiology is definitely a good model for functionally dissecting the cellular and molecular mechanisms of chronic pain.

However, due to the limitation of electrophysiology, there is still much work which remains to be done. The main unexplored field now is how to directly link the synaptic plasticity to chronic pain. Similar questions are also raised in

the field of learning and memory. In addition, more studies should focus on the mechanisms of supraspinal plasticity after chronic pain, which will not only greatly expand our knowledge of pain but also our understanding of brain functions as emotions and addition. Future studies on electrophysiology combined with genetically manipulated mice, new behavioral models and other techniques such as *in vivo*/*in vitro* imaging will provide more opportunities to identify new drug targets for treating chronic pain.

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