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Presynaptic and Postsynaptic Cortical Mechanisms of Chronic Pain

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Abstract Long-term potentiation (LTP) is a cellular model for learning and memory and believed to be critical for plastic changes in the brain. Depending on the central nervous system region, LTP has been proposed to contribute to many key physiological functions and pathological conditions, such as learning/memory, chronic pain, and drug addiction. While the induction of LTP in general requires activation of postsynaptic glutamate receptors, the expression of LTP can be mediated by postsynaptic mechanisms and/or presynaptic enhancement of glutamate release. In this review, we will evaluate recent progress made in the mechanisms of LTP in the anterior cingulate cortex (ACC) and explore its functional significance in synaptic changes after peripheral injury. Recent findings suggest that while ACC LTP in brain slice preparations is postsynaptically induced and expressed, injury triggered synaptic potentiation in the ACC contains both presynaptic enhancement of glutamate release and postsynaptic potentiation of AMPA receptor-mediated responses. Understanding presynaptic and postsynaptic mechanisms for ACC potentiation may help us to treat chronic pain in near future.

Keywords ACC · Chronic pain · LTP · Presynaptic · Postsynaptic

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Introduction

Humans possess the ability to encounter new experiences and to learn from them in order to alter future behavior and cognition. The molecular mechanisms whereby such changes are brought about have driven much of neuroscience research for the past several decades and have allowed us to understand many of the complexities of learning and plasticity [1–4]. Neurons and synapses within the central nervous system (CNS) [1] are plastic and undergo long-term changes throughout life in response to experiences and injury [5]. Long-term potentiation (LTP) of synaptic responses within the CNS has long been understood to be the mechanism that drives memory formation and storage within the brain. Essentially, it is assumed that memories are stored within the cerebral cortex as alterations of synaptic efficacy between neurons related to a particular memory [1].

Several lines of research have begun to uncover long-term alterations within the cortex in correspondence with chronic pain. Chronic pain may manifest itself through reorganization of areas related to pain transmission, modulation, and perception [6]. While it is clear that peripheral and spinal sensitization likely contribute to early phases of chronic pain, late and persistent changes in cortical regions, as well as pain-modulating brainstem regions are important for maintaining chronic pain [7–10]. Among many different models used for investigating cellular and molecular mechanisms of chronic pain, synaptic potentiation or LTP is thought to be an important model for studying synaptic potentiation after injury along nociceptive pathways [6, 11–13]. For example, LTP has been reported in synapses between primary afferent fibers and spinal dorsal horn neurons as well as in cortical synapses receiving sensory inputs [14, 15]. Some of these pain-related LTP share similar features with learning-related hippocampal LTP; however, recent studies indicate that there

are also significantly distinct molecular mechanisms involved in chronic pain but not memory [6]. In this review, we will present recent studies of pain-related cortical LTP and examine evidence linking it to chronic pain.

ACC in Pain

Several cortical areas have been implicated in the sensation of acute pain, mainly: the anterior cingulate cortex (ACC), insular cortex (IC), primary somatosensory cortex, secondary somatosensory cortex, and prefrontal cortex (PFC) [6]. The ACC, in particular, has been identified as a critical area involved in the perception of pain. The ACC is a large region around the rostrum of the corpus callosum that is active in correspondence with the presentation of painful stimuli in humans [16–19]. Electrophysiological recordings from ACC neurons have shown that they respond to noxious stimuli and in response to activity of nociceptive specific neurons [20, 21]. Neuroimaging studies have revealed that ACC activity is related to the empathy of pain, hypothesized pain, and chronic migraines [6, 22]. Studies employing warm and cold grill stimulation, where neither warm nor cold alone stimulate the ACC, have shown that the combination of warm and cold induces pain that is correlated with ACC activity [23]. Moreover, human patients with cingulotomies report an attenuation of the unpleasantness that accompanies pain [24], and animal studies have shown that lesions of the ACC result in a robust reduction of acute nociceptive responses [25, 26]. In the rat, long-term neuropathic pain was observed to decrease gray matter within the ACC, and this reduction correlated with increases in thermal and mechanical sensitivity [27], and single-digit amputation induces significant increases of immediate early genes within the ACC in minutes [28]. More recently, single-unit recordings from the ACC in conscious rats showed increases in response frequencies corresponding to increases in noxious stimuli intensities and observed large receptive fields, whereby, some ACC neurons respond to noxious stimuli on any part of the body surface [29]. Interestingly, they also observed a greater percentage of intensity-coding neurons within the somatosensory cortex compared to the ACC, whereas, a greater percentage of neurons within the ACC were involved in conditioned fear relating to noxious or painful stimuli. Together, these studies strongly suggest an important role for the ACC in the perception of chronic pain and/or pain-related fear.

Excitatory Synaptic Transmission in the ACC

Glutamate is the major fast excitatory neurotransmitter within the ACC [28, 30, 31], and various glutamate receptors including α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate (KA), *N*-methyl-D-aspartate (NMDA), and metabotropic receptors (mGluRs) have been observed within the ACC [32]. The ACC receives various direct and indirect inputs from various brain regions including the thalamus, amygdala, and hippocampus [19, 24, 28]. Local stimulation of the ACC or stimulation of thalamic projection pathways into the ACC can induce fast synaptic responses that are mediated by AMPA and KA receptors as bath applications of 6-cyano-7-nitroquinoxaline-2,3-dione have been observed to block these fast synaptic responses [30, 33]. In addition, through whole-cell patch-clamp recordings from genetically modified mice, it was demonstrated that postsynaptic KA receptors, indeed, contribute to fast synaptic transmissions of pyramidal neurons within the ACC [33]. Importantly, single-shock stimulation has been shown to induce small KA receptor-mediated excitatory postsynaptic currents (EPSCs) in the presence of picrotoxin, D-2-amino-5-phosphonopentanoic acid, and a selective AMPA receptor antagonist, GYKI 53655. Furthermore, genetic deletion of the GluR5 or GluR6 subunit significantly reduced KA EPSCs, while a deletion of both GluR5 and GluR6 can completely abolish KA EPSCs and KA-activated currents in ACC pyramidal neurons. Thus, it is quite evident that KA receptors contribute to synaptic transmission in adult ACC pyramidal neurons.

Several studies have also demonstrated NMDA receptor-mediated slow synaptic responses within the ACC [30, 34, 35] suggesting tonic activation of NMDA receptors in this region. Furthermore, in vivo recordings from freely moving mice revealed that NMDA receptors contribute to slow EPSP responses in the ACC [35]. These observations suggest that under normal physiologic conditions, most synaptic responses within the ACC are carried out by AMPA receptors, whereas, KA and NMDA receptors become active in an activity-dependent manner. Inhibitory synaptic transmission within the ACC is mediated by GABA since bath application of $GABA_A$ receptor antagonist picrotoxin completely abolished inhibitory synaptic currents.

Induction and Expression of ACC LTP

Several studies have elucidated many of the mechanisms modulating LTP within the ACC. Various stimulation protocols have been observed to induce LTP within the ACC, including theta-burst stimulation (TBS) [31], tetanic stimulation [30], paired training [36, 37], and spike-EPSP pairing [4]. Within the ACC, activation of postsynaptic glutamate NMDA receptors, as well as L-type voltage gated calcium channels results in increases of intracellular Ca^{2+}, which in turn can trigger a series of intracellular signaling cascades that contribute to LTP induction. For instance, in

vitro recordings of ACC neurons in layers V and VI display robust potentiation in response to tetanic stimulation, an effect which is blocked by the extracellular application of the NMDA receptor antagonist DL-2-amino-5-phosphonovalerate (AP-5). Similarly, in ACC slices, bath application of the NMDA receptor antagonist AP-5 blocks the induction of LTP through TBS [31]. Moreover, bath applications of either NR2A or NR2B receptor selective antagonists will only reduce LTP, while coapplication of both antagonists will completely eliminate it [4], indicating that both the NR2A and NR2B subunits of NMDA receptors are important modulators of ACC LTP. The importance of the NR2B subunit has also been confirmed in vivo. Through siRNA electroporation, Zhao and colleagues [4] were able to inhibit NR2B expression within the ACC and observed that this resulted in a marked reduction of LTP within the ACC (Fig. 1).

Postsynaptic increases in intracellular Ca^{2+} are critical for the induction of ACC LTP. Intracellular Ca^{2+} binds to calmodulin (CaM) leading to the activation of various calcium-stimulated signaling pathways [38]. Importantly, postsynaptic application of the poly-amino carboxylic acid BAPTA, a Ca^{2+} chelator, has been shown to completely abolish LTP induction within the ACC [4, 30]. In addition, selective expression within the ACC of a CaM mutant with two impaired Ca^{2+}-binding sites on the N-terminal lobe resulted in a complete block of LTP induction within ACC slices [38]. Additional intracellular signaling proteins such as Ca^{2+}-CaM stimulated adenylyl cyclases, including AC1 and AC8, as well as Ca^{2+}-CaM-dependent protein kinases, PKC, CaMKII, and CaMKIV have also been implicated in ACC LTP induction. For instance, genetic deletion AC1 completely abolishes LTP induction within the ACC through TBS, while maintaining basal glutamate transmis-

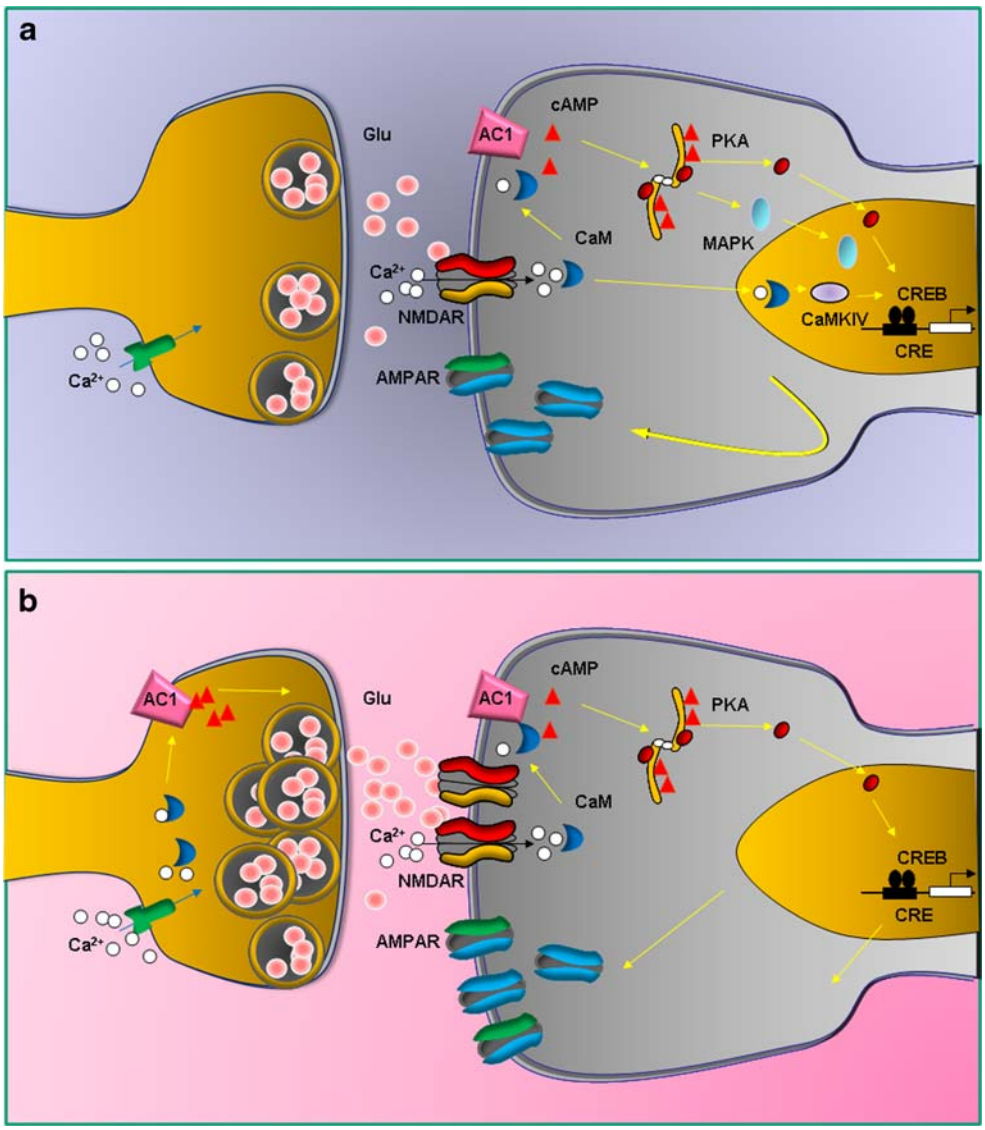

Fig. 1 Comparison of ACC LTP and cortical plastic changes in chronic pain. **a** LTP induction and expression in the ACC. In the ACC, activity triggers the release of glutamate (*Glu—pink circles*). Subsequent activation of glutamate NMDA receptors results in increases of postsynaptic Ca^{2+} in dendritic spines. Ca^{2+} is an important intracellular signal that triggers a series of biochemical events that contribute to the expression of ACC LTP. Intracellular Ca^{2+} binds to CaM and leads to the activation of calcium-stimulated ACs including AC1 and Ca^{2+}/CaM-dependent protein kinases (PKC, CaMKII, and CaMKIV). In turn, Ca^{2+}/CaM-dependent protein kinases phosphorylate glutamate AMPA receptors increase the sensitivity to extracellular glutamate. Trafficking of additional AMPA GluR1 receptors may also contribute to synaptic potentiation. **b** Possible cellular mechanism for persistent pain. Inflammatory or nerve injuries lead to presynaptic and postsynaptic changes within ACC synapses. Presynaptic enhancements of glutamate release, as well as postsynaptic alterations in AMPA and NMDA receptor-mediated responses contribute to enhanced noxious sensory transmission within the brain

sion [31]. Similarly, a robust reduction in cyclic AMP response element binding protein (CREB) activity has been observed in CaMKIV knockout mice suggesting that CaMKIV, a kinase predominantly expressed in the nuclei, modulates CREB activity [39]. Furthermore, it has been demonstrated that in brain slices of CaMKIV knockout mice, TBS stimulation failed to induce LTP within the ACC, whereas, wild type mice displayed a robust level of LTP under this protocol [40]. In addition, activation of AC1 and AC8 leads to the activation of PKA, as well as CREB, and in turn, CREB and other immediate early genes such as c-Fos and Egr1 activate targets that are thought to lead to structural changes [41]. Interestingly, genetic deletion of Egr1 significantly attenuates LTP within the ACC [41] implicating that intracellular protein synthesis may play a role in ACC LTP. More recently, the fragile X mental retardation protein (FMRP) has also been implicated in ACC LTP. FMRP is located in dendritic spines and appears to play a role in local protein synthesis [43]. The paired training protocol, which induces a robust potentiation of ACC synapses, failed to potentiate ACC synapses of FMR1 KO mice [4]. More recently, we demonstrated that activation of group I mGluRs results in upregulation of FMRP in ACC neurons of adult mice through Ca^{2+}-dependent signaling pathways [7]. Using genetic approaches, we found that AC1 and CaMKIV contribute to this upregulation. It is, thus, evident that postsynaptic Ca^{2+} elevation is crucial for the induction of ACC LTP and that a host of intracellular signaling molecules contribute to these effects.

A critical feature of ACC LTP expression is the functional recruitment of postsynaptic AMPA receptors. Phosphorylation of postsynaptic AMPA receptors is driven through Ca^{2+}-CaM-dependent protein kinases and results in an increase of postsynaptic sensitivity to glutamate release. For example, the AMPA receptor subunit GluR1 appears to play a critical role in ACC LTP expression, as postsynaptic preapplication of the GluR1 inhibiting peptide Pep1-TGL completely blocks LTP induction by paired training (Fig. 1) [37]. The involvement of the GLuR1 subunit appears to be time-dependent as the LTP blocking effect of Pep1-TGL did not occur if the antagonist was introduced 5 min after pairing protocol commenced, suggesting that the functional recruitment of AMPA receptor GluR1 subunits is complete within 5–10 min.

Chronic Pain Causes Plastic Changes in the ACC: In Vitro

Postsynaptic Mechanisms

Two types of animal model for chronic injuries are commonly employed to investigate the cellular and molecular mechanisms underlying chronic pain. Inflammatory chronic pain models use subcutaneous injections into the hindpaw of formalin or complete Freund's adjuvant (CFA), resulting in tissue injury. Neuropathic pain models on the other hand are commonly caused through surgical injury of a peripheral nerve. These have proven to be robust models for the study of chronic pain and have begun to yield much insight into the molecular mechanisms of chronic pain. In vitro observations, in conjunction with these models, have shown that the NMDA NR2B subunit within the ACC appears to play a decisive role in chronic pain development. For instance, in transgenic mice with forebrain-targeted NR2B overexpression, an enhanced inflammatory pain response was observed, while behavioral responses to acute pain were unaltered [44]. Specifically, transgenic mice exhibited enhanced behavioral responses to formalin injections that corresponded with an upregulation of immediate early genes within pain-related areas including the ACC, somatosensory cortex, and IC compared to control type mice. More recently, it was shown that peripheral tissue inflammation caused by CFA resulted in an upregulation of NR2B receptors within the ACC [35]. Specifically, in vitro electrophysiological recordings revealed increases in NMDA mediated EPSCs in CFA treated mice. Importantly, application of the selective NR2B antagonist Ro-25-6981 reduced EPSC amplitude to a greater extent in CFA versus saline-treated mice. Western blot analysis confirmed upregulation of NR2B expression within the ACC, while no increases were detected of the NR1 or NR2A subtypes. Additionally, they also demonstrated that local ACC microinjections of NR2B receptor selective antagonists attenuated behavioral allodynia. Thus, in contrast to the hippocampus, NR2B receptor activity during central plasticity within the ACC appears to be robust (Table 1).

Additional postsynaptic alterations have been observed in correspondence to chronic pain. In a recent study,

Table 1 Comparison of synaptic mechanisms for ACC LTP and chronic pain

	Induction	Expression or maintenance	Keysignaling molecules
LTP	NMDA receptor: NR2A and NR2B	Postsynaptic GluR1 GluR1 (GRIA1)upregulation	AC1,cAMP, CaMKIV, Egr1, CREB
Neuropathic pain	NMDA receptor	Presynaptic enhancement of glutamate release Postsynaptic GluR1 (GRIA1)upregulation	AC1, cAMP
Inflammatory pain	NMDA receptor	Presynaptic enhancement of glutamate release Postsynaptic NR2B upregulation	AC1, AC8, cAMP, Egr1

western blot analysis revealed an upregulation of phosphorylated GluR1 within the ACC in a mouse peripheral nerve injury model that corresponded with increases in postsynaptic AMPA receptor-mediated responses [32]. Similarly, amputation or even strong peripheral stimulation of the hindpaw in rats induces activation of plasticity-related immediate early genes, including Egr1, CREB, and c-Fos within ACC neurons [28, 40]. In addition, AC1 and AC8 appear to modulate behavioral sensitization in response to chronic pain. Using AC1 and AC8 knockout mice, Wei and colleagues [40] observed a marked reduction in behavioral nociceptive responses to formalin injection on the hindpaw. Interestingly, this reduction was even greater in AC1/AC8 double knockout mice. Furthermore, AC1 knockout and AC1/AC8 double knockout mice displayed a robust reduction in behavioral allodynia in response to a CFA injection of the hindpaw. This effect was not observed in AC8 knockout mice (Table 1).

Presynaptic Mechanisms

Recently, it has been demonstrated that presynaptic release of excitatory synaptic transmission within the ACC is enhanced by peripheral nerve injury. Employing a neuropathic pain model ligating the common peroneal nerve, it was observed that the presynaptic release probability of glutamate was enhanced within layer II/III neurons after injury [32]. Similarly, enhanced presynaptic neurotransmitter release has been observed in ACC synapses of mice with chronic inflammatory pain [36, 45]. Specifically, an increased frequency of AMPA receptor-mediated mini-EPSCs (mEPSCs) was detected in mice injected with CFA [36]. Importantly, no alterations in amplitude, decay kinetics, or current–voltage relationships were observed in AMPA mediated mEPSCs indicating that the frequency alterations were a result of increases in transmitter release. More recently, quantal analysis confirmed increases in presynaptic neurotransmitter release probability in mice suffering from neuropathic pain [45]. Through comparisons of mice exposed to a peripheral nerve ligation model with those exposed to CFA inflammation, it was shown that both the probability of transmitter release and the number of available vesicles increased in correspondence with CFA induced peripheral inflammation, whereas, only the probability of transmitter release, but *not* the number of available vesicles, increased in response to neuropathic pain. Thus, although chronic inflammatory and neuropathic pain manifest through presynaptic and postsynaptic mechanisms, presynaptic mechanisms for enhanced excitatory transmission appear to differ. Interestingly, a postsynaptic role for AMPA GluR1 receptor has only been observed in peripheral nerve injury models, but has yet to be confirmed for chronic inflammatory pain. Nonetheless, they share robust similarities in the nociceptive behavior they evoke and overlap on some key potentiation mechanisms. Thus, it is important for future research to extract the subtle differences in the chronic pain mechanisms that are altered from inflammatory and peripheral injuries to better understand how and why chronic pain develops (Table 1).

Chronic Pain Causes Plastic Changes in the ACC: In Vivo

Recent in vivo observations provide evidence that peripheral injury triggers synaptic activity within the ACC and related cortical areas. For instance, through the use of inflammatory and neuropathic pain models in addition to different LTP inducing protocols, studies have identified changes in cortical LTP that correspond with chronic and neuropathic pain. In vivo local field ACC recordings of anesthetized rats revealed that digit amputation leads to rapid enhancement of sensory responses to peripheral electrical shocks of the intact paw [21]. Moreover, this enhancement corresponded with long-lasting potentiation of field excitatory postsynaptic potentials (EPSPs) that lasted for over 120 min. Similarly, field EPSPs produced by focal cortical stimulation within the ACC were also potentiated after digit amputation suggesting that long-lasting potentiation occurred locally within the ACC. Importantly, blocking of peripheral inputs with QX-314 120 min after amputation did not alter the responses observed in the ACC indicating that the potentiation, indeed, occurred at a cortical level, and is not due to ongoing peripheral activity. It is, thus, possible that peripheral injury induces chronic pain via the potentiation of synaptic responses within the ACC. More recently, peripheral nerve injury in rats was shown to yield morphological changes within the medial prefrontal cortex (mPFC), an area that includes the ACC [45]. Specifically, basal, but not apical, dendrites of injured rats displayed increases in spine density and were longer and with more branches than those exposed to a sham surgery. Furthermore, a linear correlation was observed between the NMDA/AMPA ratio of pyramidal synaptic currents and the nociceptive thresholds of the injured rats, suggesting a possible relationship between synaptic transmission within the mPFC/ACC and the perception of chronic pain.

Future Directions

In vitro and in vivo observations provide compelling evidence for the involvement of both presynaptic and postsynaptic mechanisms in the development of chronic pain. The postsynaptic events that have been demonstrated

to accompany animal models of chronic pain can be at least partially mimicked by experimentally induced LTP in ACC in vitro slices. The presynaptic observations on the other hand are less understood and a new presynaptically mediated ACC LTP is clearly needed.

The expression of LTP within the ACC provides a robust methodological approach towards the study of cortical LTP in general. Although learning and the development of chronic pain appear to share common molecular mechanisms, it is unlikely that these are the only facets of life brought about through LTP. It is far more likely that LTP represents a synaptic language, whereby, neurons reorganize and become more efficient, allowing for a rich repertoire of what is human behavior. Of special note is that chronic pain has been observed to interfere with learning. Through subcutaneous hind paw injections of CFA, Zhao and colleagues [36] identified a trace fear memory deficit in mice expressing inflammatory pain. It would be of interest if other conditions implicated to induce LTP interfere with learning. Studies of human stroke patients, musicians, or athletes may yield some answers. Although much progress remains to be done, the identification of LTP within an increasing number of cortical areas and in an increasing number of pathological conditions adds credence to its value as a molecular substrate for CNS plasticity. Further characterizations of LTP within these various facets will hopefully allow us to decipher the language of the human brain.

References

1. Bliss T, Collingridge G (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 361:31–39
2. Nicoll R, Malenka R (1995) Contrasting properties of two forms of long-term potentiation in the hippocampus. Nature. 377:115–118
3. Kandel E (2001) Nobel lecture. The molecular biology of memory storage: a dialog between genes and synapses. Biosci Rep 21 (5):565–611
4. Zhao M-G, Toyoda H, Lee Y-S, Wu L-J, Ko SW, Zhang X-H et al (2005) Roles of NMDA NR2B subtype receptor in prefrontal long-term potentiation and contextual fear memory. Neuron 47 (6):859–872
5. Kaas JH, Qi H-X, Burish MJ, Gharbawie OA, Onifer SM, Massey JM (2008) Cortical and subcortical plasticity in the brains of humans, primates, and rats after damage to sensory afferents in the dorsal columns of the spinal cord. Exp Neurol 209(2):407–416
6. Zhuo M (2008) Cortical excitation and chronic pain. Trends Neurosci 31(4):199–207
7. Wang H, Wu L-J, Kim SS, Lee FJS, Gong B, Toyoda H et al (2008) FMRP acts as a key messenger for dopamine modulation in the forebrain. Neuron 59(4):634–647
8. Gebhart GF (2007) It's chickens and eggs all over again: is central reorganization the result or cause of persistent visceral pain? Gastroenterology 132(4):1618–1620
9. Porreca F, Ossipov M, Gebhart G (2002) Chronic pain and medullary descending facilitation. Trends Neurosci 25(6):319–325
10. Zhang H-M, Qi Y-J, Xiang X-Y, Zhang T, Liu X-G (2001) Time-dependent plasticity of synaptic transmission produced by long-term potentiation of C-fiber evoked field potentials in rat spinal dorsal horn. Neurosci Lett. 315:81–84
11. Sandkuhler J (2007) Understanding LTP in pain pathways. Molecular Pain 3(1):9
12. Gautam Bhave, Robert W. Gereau IV (2004) Posttranslational mechanisms of peripheral sensitization. J Neurobiol 61(1):88–106
13. Woolf CJ, Salter MW (2000) Neuronal plasticity: increasing the gain in pain. Science 288(5472):1765–1768
14. Glazewski S, Fox K (1996) Time course of experience-dependent synaptic potentiation and depression in barrel cortex of adolescent rats. J Neurophysiol 75(4):1714–1729
15. Randic M, Jiang M, Cerne R (1993) Long-term potentiation and long-term depression of primary afferent neurotransmission in the rat spinal cord. J Neurosci. 13:5228–5241
16. Davis KD, Taylor SJ, Crawley AP, Wood ML, Mikulis DJ (1997) Functional MRI of pain- and attention-related activations in the human cingulate cortex. J Neurophysiol 77(6): 3370–3380
17. Derbyshire SWG, Vogt BA, Jones AKP (1998) Pain and stroop interference tasks activate separate processing modules in anterior cingulate cortex. Exp Brain Res 118(1):52–60
18. Lenz FA, Rios M, Zirh A, Chau D, Krauss G, Lesser RP (1998) Painful stimuli evoke potentials recorded over the human anterior cingulate gyrus. J Neurophysiol 79(4):2231–2234
19. Vogt BA, Vogt L, Farber NB, Bush G (2005) Architecture and neurocytology of monkey cingulate gyrus. J Comp Neurol 485 (3):218–239
20. Hutchison W, Davis K, Lozano A, Tasker R, Dostrovsky J (1999) Pain-related neurons in the human cingulate cortex. Nat Neurosci 2(5):403–405
21. Wei F, Zhuo M (2001) Potentiation of sensory responses in the anterior cingulate cortex following digit amputation in the anaesthetised rat. J Physiol 532(Pt 3):823–833
22. Johansen J, Fields H (2004) Glutamatergic activation of anterior cingulate cortex produces an aversive teaching signal. Nat Neurosci 7(4):398–403
23. Craig A, Reiman E, Evans A, Bushnell M (1996) Functional imaging of an illusion of pain. Nature. 384:258–260
24. Zhuo M (2006) Molecular mechanisms of pain in the anterior cingulate cortex. J Neurosci Res 84(5):927–933
25. Johansen J, Fields H, Manning B (2001) The affective component of pain in rodents: direct evidence for a contribution of the anterior cingulate cortex. Proc Natl Acad Sci USA 98(14):8077–8082
26. Chen D, Ho S, Liang K (2000) Startle responses to electric shocks: measurement of shock sensitivity and effects of morphine, buspirone and brain lesions. Chin J Physiol 43(1):35–47
27. Seminowicz DA, Laferriere AL, Millecamps M, Yu JSC, Coderre TJ, Bushnell MC (2009) MRI structural brain changes associated with sensory and emotional function in a rat model of long-term neuropathic pain. Neuroimage 47(3):1007–1014
28. Wei F, Li P, Zhuo M (1999) Loss of synaptic depression in mammalian anterior cingulate cortex after amputation. J Neurosci 19(21):9346–9354
29. Kuo C-C, Chiou R-J, Liang K-C, Yen C-T (2009) Differential involvement of the anterior cingulate and primary sensorimotor cortices in sensory and affective functions of pain. J Neurophysiol 101(3):1201–1210
30. Sah P, Nicoll RA (1991) Mechanisms underlying potentiation of synaptic transmission in rat anterior cingulate cortex in vitro. J Physiol 433(1):615–630
31. Liauw J, Wu L-J, Zhuo M (2005) Calcium-stimulated adenylyl cyclases required for long-term potentiation in the anterior cingulate cortex. J Neurophysiol 94(1):878–882

32. Xu H, Wu L-J, Wang H, Zhang X, Vadakkan KI, Kim SS et al (2008) Presynaptic and postsynaptic amplifications of neuropathic pain in the anterior cingulate cortex. J Neurosci 28(29):7445–7453
33. Wu L, Zhao M, Toyoda H, Ko S, Zhuo M (2005) Kainate receptor-mediated synaptic transmission in the adult anterior cingulate cortex. J Neurophysiol 94(3):1805–1813
34. Liauw J, Wang G, Zhuo M (2003) NMDA receptors contribute to synaptic transmission in anterior cingulate cortex of adult mice. Sheng Li Xue Bao 55(4):373–380
35. Wu L, Toyoda H, Zhao M, Lee Y, Tang J, Ko S et al (2005) Upregulation of forebrain NMDA NR2B receptors contributes to behavioral sensitization after inflammation. J Neurosci 25 (48):11107–11116
36. Zhao M, Ko S, Wu L, Toyoda H, Xu H, Quan J et al (2006) Enhanced presynaptic neurotransmitter release in the anterior cingulate cortex of mice with chronic pain. J Neurosci 26 (35):8923–8930
37. Toyoda H, Wu L, Zhao M, Xu H, Zhuo M (2007) Time-dependent postsynaptic AMPA GluR1 receptor recruitment in the cingulate synaptic potentiation. Dev Neurobiol 67(4):498–509
38. Wei F, Xia X, Tang J, Ao H, Ko S, Liauw J et al (2003) Calmodulin regulates synaptic plasticity in the anterior cingulate cortex and behavioral responses: a microelectroporation study in adult rodents. J Neurosci 23(23):8402–8409
39. Zhuo M (2007) Neuronal mechanism for neuropathic pain. Molecular Pain 3(1):14
40. Wei F, Qiu C-S, Kim SJ, Muglia L, Maas JW Jr, Pineda VV et al (2002) Genetic elimination of behavioral sensitization in mice lacking calmodulin-stimulated adenylyl cyclases. Neuron 36(4):713–726
41. Zhuo M (2005) Canadian association of neuroscience review: cellular and synaptic insights into physiological and pathological pain. Can J Neurol Sci 32:27–36
42. Ko SW, Vadakkan KI, Ao H, Gallitano-Mendel A, Wei F, Milbrandt J et al (2005) Selective contribution of Egr1 (zif/268) to persistent inflammatory pain. J Pain 6(1):12–20
43. Bagni C, Greenough WT (2005) From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. Nat Rev Neurosci 6(5):376–387
44. Wei F, Wang G, Kerchner G, Kim S, Xu H, Chen Z et al (2001) Genetic enhancement of inflammatory pain by forebrain NR2B overexpression. Nat Neurosci 4(2):164–169
45. Toyoda H, Zhao M, Zhuo M (2009) Enhanced quantal release of excitatory transmitter in anterior cingulate cortex of adult mice with chronic pain. Molecular Pain. 5:4
46. Metz AE, Yau H-J, Centeno MV, Apkarian AV, Martina M (2009) Morphological and functional reorganization of rat medial prefrontal cortex in neuropathic pain. Proc Natl Acad Sci 106 (7):2423–2428