

# Erasing injury-related cortical synaptic potentiation as a new treatment for chronic pain

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**Abstract** Synaptic plasticity in the spinal cord and the cortex is believed to be important for the amplification of painful information in chronic pain conditions. The investigation of molecular mechanism responsible for maintaining injury-related plastic changes, such as through the study of long-term potentiation in these structures, provides potential novel targets for designing new medicine for chronic pain. Recent studies using integrative neurobiological approaches demonstrate that protein kinase M zeta (PKM $\zeta$ ) maintains pain-induced persistent changes in the anterior cingulate cortex (ACC), and inhibiting PKM $\zeta$  by  $\zeta$ -pseudosubstrate inhibitory peptide produces analgesic effects in animal models of chronic pain. We propose that targeting PKM $\zeta$ , or its up- or downstream signaling proteins, in the ACC may provide novel clinical treatment for chronic pain.

**Keywords** Pain · Synapse · Brain

## Introduction

Activity-dependent synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD) is a key neuronal function for the brain. To potentiate or depress synaptic responses in response to incoming inputs from the periphery allow animals, including humans, to “learn” new information, and “store” important messages in central synapses. Under physiological conditions, such central plasticity is believed to contribute to the ability to adapt to the natural environment. To investigate such mechanisms and thereby to discover drugs to enhance such ability is critical for us to treat various forms of dementia and mild cognitive impairment and to improve cognitive capacity. However, recent cumulative evidence suggests that the brain may employ similar plastic mechanisms to handle injury information. Under pathological conditions, synaptic plasticity acts as positive amplification to exaggerate disease-related conditions. Chronic pain is a typical example of such central amplification.

Chronic pain is a major health issue and is mainly caused by tissue or nerve injury. In addition to spontaneous pain, there are two common pathological conditions that develop after tissue or nerve injury: allodynia and hyperalgesia. In allodynia, there is a reduction in pain threshold, and consequently, non-noxious stimuli that normally do not cause pain now induce pain. In hyperalgesia, there is an enhanced response to noxious stimuli. Peripheral sensitization and central plasticity are likely to contribute to chronic pain. Major focuses are at peripheral and spinal plastic changes, since these two areas are the best targets for medical manipulation or treatment for chronic pain with

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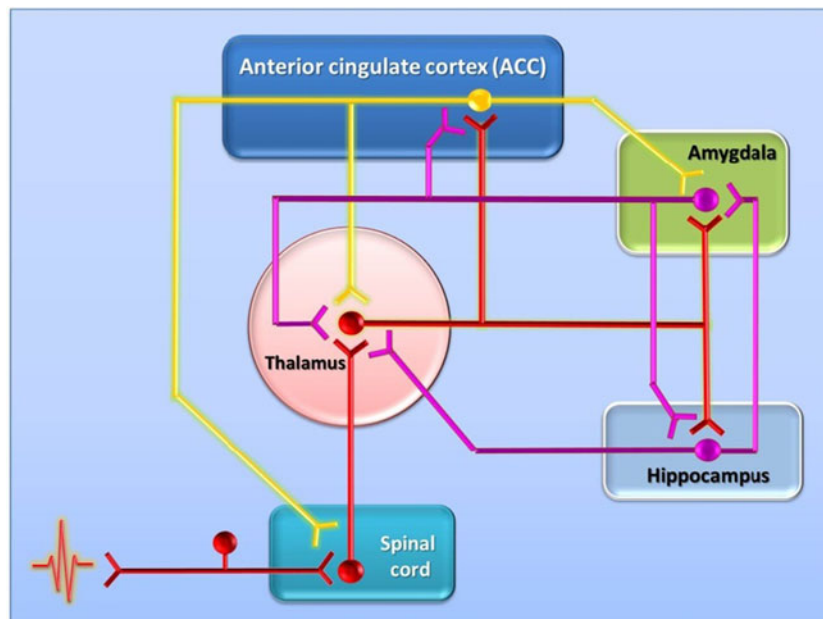
less or no central side effects. However, increasing evidence suggest cortical plasticity also takes place right after the injury; and some plastic changes may even occur at cortical levels without the requirement of continuous spinal or peripheral activity (Fig. 1). Here, we will review recent progress made related to injury-triggered cortical plasticity and explore possible molecular mechanism for maintaining LTP. We suggest that understanding these molecular mechanisms will provide new insights for treating chronic pain in the future.

### LTP as a model for synaptic learning

LTP is the most extensively studied physiological process by those interested in understanding the molecular and cellular basis of learning and memory in vertebrates [1]. LTP is a widespread phenomenon exhibited by most excitatory synapses so far investigated, where it encodes regionally relevant information such as spatial learning in the hippocampus and persistent pain in the anterior cingulate cortex (ACC). It is well established that most forms of LTP are induced by the transient activation of *N*-methyl-D-aspartate receptors (NMDARs), though there are

some NMDAR-independent forms of LTP in the central nervous system (CNS). Upon NMDAR activation, there is a transient elevation of  $\text{Ca}^{2+}$  in the activated postsynaptic spine, which triggers a signaling cascade involving a variety of protein kinases, such as  $\text{Ca}^{2+}$ /calmodulin (CaM)-dependent protein kinase II (CaMKII), protein kinase A (PKA), and mitogen-activated protein kinase (MAPK), as well as other signaling molecules. LTP is expressed by a variety of different mechanisms that include a long-lasting enhancement in L-glutamate release, alterations in postsynaptic voltage-dependent conductance, and changes in NMDARs and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA), the receptors that mediate most fast synaptic transmission in the CNS.

With respect to AMPARs, there is evidence for changes in both their single channel conductance properties and in their number that are expressed in the postsynaptic membrane [2]. An increase in single channel conductance could be due to a number of factors, including (a) a higher L-glutamate concentration in the vicinity of the receptors, (b) an alteration in phosphorylation, such as of Ser845 of the GluA1 subunit, or (c) an alteration in subunit composition, caused by the rapid exchange of  $\text{Ca}^{2+}$ -impermeable (i.e., GluA2 containing) with  $\text{Ca}^{2+}$ -permeable



**Fig. 1** A model of sensory circuits for pain transmission and modulatory in the central nervous system. Under normal physiological conditions, noxious information is sent to the spinal cord, and some spinal cord neurons send projection terminals to supraspinal structures. Neurons in the thalamus play key roles in relaying most of these ascending sensory inputs. The cortical areas, including the ACC, IC, S1, S2, and PFC, are activated and contribute to different aspects of pain perception. Neurons in the hippocampus are also activated and can contribute to the formation of pain-related spatial memory and mood responses. Activation of the amygdala (as well as the ACC) also

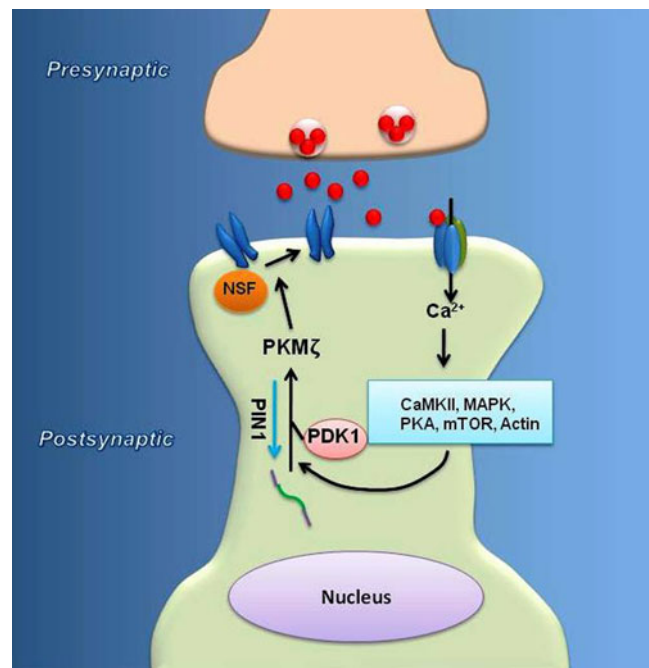
contributes to pain-related fear memory and pain modulation. As protective responses to noxious stimuli, descending facilitatory systems can also be activated. Facilitated spinal nociceptive transmission might trigger faster escape responses. In the case of an inescapable situation, the descending inhibitory system can be activated, and the neurotransmitters such as serotonin (5-HT) and norepinephrine (NE) will be released from descending projection fibers in the dorsal horn of the spinal cord. They then inhibit dorsal horn synaptic transmission

(i.e., GluA2 lacking) AMPARs. AMPAR number could be altered via a variety of mechanisms, though it is likely to involve a two-component mechanism that involves (a) insertion of AMPARs into the plasma membrane followed by (b) their lateral diffusion into the postsynaptic membrane. AMPARs are then stabilized within the synapse with an interaction with *N*-ethylmaleimide sensitive factor (NSF) [3].

In addition to a long-lasting enhancement of synaptic transmission, information can also be stored by the long-lasting decrease in synaptic transmission, via the process of LTD. LTD is often also triggered by the synaptic activation of NMDARs, using different patterns of activation and involving different transduction mechanisms from LTP. A second major form of LTD can be triggered via the activation of metabotropic glutamate receptors.

### Protein kinase M zeta and the maintenance of LTP

So far, there have been numerous studies to understand the molecular mechanisms of initiation and acquisition of LTP, primarily studied in the hippocampus. Accordingly, now we know several of the signaling molecules that are implicated in these stages. However, less is known about how LTP is maintained and thus needs to be elucidated. Recently, Todd C. Sacktor and his colleagues have revealed that protein kinase M zeta (PKM $\zeta$ ) is one of the key factors in maintaining LTP [4–6]. Originally, PKM $\zeta$  was considered to be a cleavage product of protein kinase C zeta (PKC $\zeta$ ) [4, 7]. However, it was reported that PKM $\zeta$  is produced from its own mRNA transcript, distinct from PKC $\zeta$  synthesis [8]. Usually, most protein kinases consist of two components; regulatory and catalytic domains or subunits. However, PKM $\zeta$  contains the same catalytic domain as PKC $\zeta$ , yet without the regulatory component. This unique feature confers constitutive catalytic activity upon PKM $\zeta$  although it requires phosphorylation by phosphoinositide-dependent protein kinase 1 (PDK1; Fig. 2) [9, 10]. The PKM $\zeta$  could be an attractive candidate for sustaining synaptic strength because of its persistent activation mode. Previous studies suggested the possibility that PKM $\zeta$  is a key factor in maintaining late LTP [4]. Among PKC isoforms, only PKM $\zeta$  increases during LTP maintenance phase in hippocampal slices. Although other isoforms of PKC are enhanced immediately during the induction phase of LTP initiated by single tetanic stimulation, their expression returns to the basal level within 30 min after tetanus. More direct evidence showing the role of PKM $\zeta$  came from pharmacological studies with a PKM $\zeta$  inhibitor— $\zeta$  pseudosubstrate inhibitory peptide (ZIP). The application of ZIP during LTP maintenance phase



**Fig. 2** A simple model for the role of PKM $\zeta$  in hippocampal LTP. In the hippocampus, activities trigger the release of glutamate, the activations of NMDARs result in the influx of Ca<sup>2+</sup>, which then triggers several Ca<sup>2+</sup>-related signaling pathway. Especially, for the late-phase LTP (L-LTP), PKA activity is important. Activated PKA and MAPK release the translational blocker, and PKM $\zeta$  mRNA is translated and further phosphorylated by PDK1. The activating PKM $\zeta$  potentiate the AMPAR responses by increasing the possible number of the GluA2 containing AMPARs through the action of *N*-ethylmaleimide-sensitive factor (NSF)

in tetanized slices reverses the enhanced synaptic strength [5, 6]. This phenomenon was shown in vivo system as well [11, 12].

Since PKM $\zeta$  was identified as one of the factors maintaining LTP, many attempts have been made to examine whether PKM $\zeta$  has a role in the maintenance of long-term memory because LTP has been considered as a synaptic mechanism of memory. Using the PKM $\zeta$  inhibitor ZIP, it has been reported that PKM $\zeta$  is indeed required for the maintenance of several types of memory in various brain regions. For example, in the hippocampus, PKM $\zeta$  is necessary for sustaining spatial information (place avoidance task, radial arm maze, water maze) and fear memory (trace eyeblink conditioning) [11–13]. When PKM $\zeta$  is inhibited in the amygdala, cued fear memory, contextual fear memory, and inhibitory avoidance memory are erased during the examined time [13–16]. In cortical regions, persistent PKM $\zeta$  activity is involved in the storage of long-term memory as well. It has been shown that ZIP infusion into the insular cortex eliminates the memory of conditioned taste aversion [17, 18]. Recently, it has been further demonstrated that overexpression of PKM $\zeta$  in the insular cortex enhanced long-term memory, whereas a dominant negative PKM $\zeta$

disrupted memory [19]. Moreover, emotional fear memory was impaired by ZIP infusion into the secondary sensory cortices [20]. Besides rodent studies, PKM $\zeta$  is found in the head of *Drosophila* and has a similar role related to memory maintenance in rodents [21].

### LTP in pain-related central synapses

Unlike hippocampal LTP, the studies of LTP in pain-related synapses were slow in the beginning. In the dorsal horn of the spinal cord, it is not easy to obtain field recordings of synaptic responses. Due to the mixed neuronal populations, tetanic stimulation of the dorsal root afferent fibers often leads to LTP or LTD in different cells recorded. The use of whole-cell patch recording technique, especially recording from retrograde-labeled projecting cells, significantly improves our understanding of spinal cord LTP [22, 23].

Several cortical areas including the ACC and insular cortex have been reported to contribute to chronic pain. Genetic, pharmacological, and electrophysiological approaches have been used to investigate the basic mechanisms for LTP at ACC synapses [24, 25]. Different stimulation protocols can be used for inducing LTP in ACC pyramidal cells. A conventional pairing protocol (synaptic activity paired with postsynaptic depolarization), a spike-excitatory postsynaptic potential pairing protocol, and a theta burst stimulation (TBS) protocol all induce LTP in ACC pyramidal neurons. The activation of NMDARs is critical for the induction of ACC LTP [24]. Blocking LTP in the ACC by microinjection of a NMDA receptor antagonist AP-5 suppresses injury-induced allodynia [26]. Ca<sup>2+</sup>-stimulated, neuron-specific adenylyl cyclase subtype 1 (AC1) is highly expressed in the ACC neurons [26], and LTP requires the activation of AC1 activity as demonstrated by the use of gene knockout mice lacking AC1 [27] or a selective inhibitor of AC1 [28]. For the expression of LTP, AMPARs containing the GluA1 subunit are involved. For example, ACC LTP is absent GluA1 knockout mice [29].

LTP has also been reported in other pain-related cortical areas. These include prefrontal cortex, somatosensory cortex area, and insular cortex. However, the exact molecular mechanisms for the induction and expression of LTP are mostly unknown and remain to be investigated in future studies. Recent studies have indicated that amygdala synapses undergo plastic changes after injury [30]. Different forms of amygdala LTP have been reported, mainly in the areas related to the study of mechanisms of fear memory. It is possible that some of the synaptic potentiation may be shared by chronic pain and emotional fear. Future studies are clearly needed to determine any distinct changes related to chronic pain.

### ACC and chronic pain

It has been known that the ACC is important for mediating the emotional and the attentive responses to internal and external stimulation [31–33]. Brain imaging studies provide direct evidence that several limbic areas can be activated by peripheral pain stimulation. Among them, the ACC is found to be the most reliable area, which can respond to different noxious or painful stimuli [34, 35]. In vivo and in vitro electrophysiological experiments have demonstrated that the ACC neurons respond to peripheral somatosensory or visceral nociceptive stimulation in various types of animals, such as mouse, rat, rabbit, and monkey [28, 36–42]. The ACC may exert a top-down modulation on spinal nociceptive transmission through the descending regulatory systems, since electrical and chemical stimulation of the ACC facilitated behavioral responses to noxious stimulation [34, 43, 44]. While lesions of the ACC significantly reduced the animal's sensitivity to noxious stimuli [45], inhibiting glutamatergic activity by injection of 6-cyano-7-nitroquinoxaline-2,3-dione into the ACC reduced the mechanical allodynia and pain-related vocalization [46, 47]. Consistent with animal studies, clinical studies found that frontal lobotomies or cingulotomies abolished the unpleasantness of pain in patients [48, 49]. Furthermore, unlike the somatosensory cortex, it has been proposed that the ACC may not code information for the location, intensity and duration of peripheral stimuli, but rather contributes to the affective content or the unpleasantness of pain [33, 34, 41]. It has been proved that, in freely moving animals, stimulation of the ACC also produced aversive behaviors or fear responses, which are typical emotional reactions related to pain [44, 50].

### ACC LTP after injury: pre- and postsynaptic mechanisms

Different approaches have been employed to investigate the changes of cingulate synaptic transmission under chronic pain condition. It has been found that synaptic transmission in the ACC was enhanced by periphery inflammation [51], nerve injury [37, 46], and digit amputation [52]. This enhancement shares similar neuronal mechanism with LTP because it occluded the plasticity of synaptic transmission in the ACC. For example, the amputation of a third hindpaw digit in adult rats caused loss of LTD, which could last for at least 2 weeks [52]. Recently, we examined the effects of nerve injury on LTP induction in the ACC and found that the L-LTP induced by TBS from nerve injury mice was significantly smaller than that from sham or naïve mice [37]. In addition, inhibiting the enhanced glutamatergic synaptic transmission decreased peripheral allodynia [46].

The enhancements of synaptic transmission induced by chronic pain in the ACC were mediated by both pre- and postsynaptic mechanisms [34]. It was found that glutamate release was increased by chronic pain. For example, it has been reported that paired-pulse facilitation was decreased and that the frequency of miniature EPSCs was increased by periphery inflammation [51], and similar results were observed under neuropathic pain condition [46]. Further experiments found that AC1 was involved in the modulation of the presynaptic release of glutamate [53, 54].

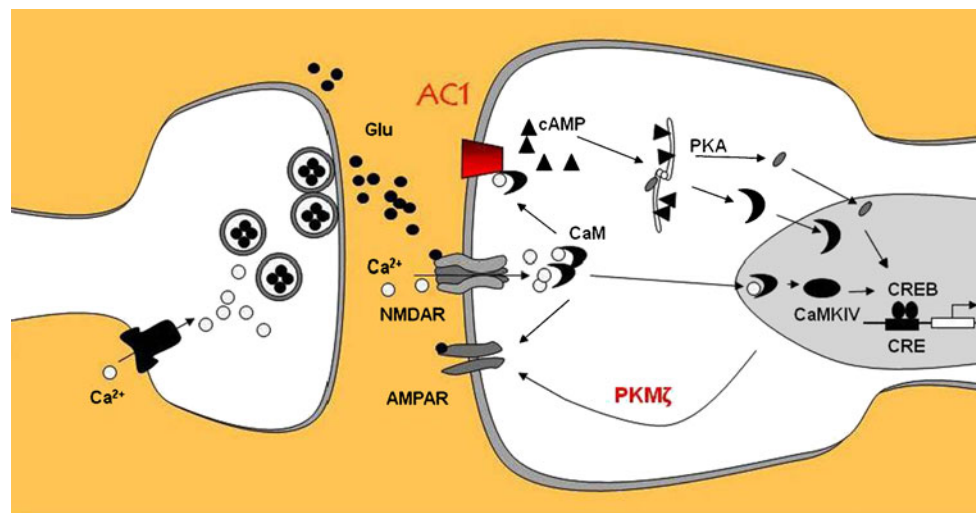
AMPA receptors, kainate receptors, and NMDARs are the major targets of glutamate in the ACC [55]. Integrative evidences showed that the postsynaptic components are also involved in the enhancement of synaptic transmission. The expression of AMPARs in the postsynaptic sites was increased under chronic pain condition [46]. This was further confirmed for the ACC neurons activated by allodynia by using the FosGFP transgenic mice, in which the expression of green fluorescent protein is controlled by the promoter of the *c-fos* gene [37]. Calcium signaling pathway in the postsynaptic cells may be involved in the initiation of LTP-like change induced by chronic pain. It was found that  $Ca^{2+}$  elevation in the postsynaptic sites was critical for the LTP induction in the ACC.  $Ca^{2+}/CaM$  leads to the activation of calcium-stimulated signaling pathways [56, 57]. In turn,  $Ca^{2+}/CaM$  can stimulate the activities of AC1, which can convert adenosine-5'-triphosphate to a second messenger cyclic adenosine monophosphate (cAMP). AC1 is critical for the induction of LTP in the ACC. Using a new selective AC1 inhibitor, we found that inhibiting of cingulate AC1 significantly reduced mechan-

ical allodynia induced by nerve injury [27, 28]. The increased cAMP further activates PKA-dependent signaling pathway, including MAPK [58] and cAMP response element-binding protein (CREB).  $Ca^{2+}/CaM$  can also activate different forms of CaM kinases, among them, CaMKIV is distinguished in its capacity to activate CREB-dependent transcription (Fig. 3) [59].

### Molecular regulation of PKM $\zeta$ in chronic pain

Painful stimulation on the periphery induces LTP-like change, especially in the ACC [52]. This implies that sustained LTP in the ACC maybe at least partially responsible for chronic pain induced by peripheral nerve injury. How is LTP maintained in the ACC? We recently showed that PKM $\zeta$  is necessary for maintaining LTP in the ACC [37], like in the hippocampus. Calcium-dependent cAMP-PKA signaling pathway acts upstream of PKM $\zeta$ . In *AC1* knockout mice, nerve injury neither induced hyperalgesia nor increased PKM $\zeta$  and phosphorylated PKM $\zeta$  (p-PKM $\zeta$ ) levels in the ACC. Moreover, elevated cAMP level after forskolin treatment enhances both PKM $\zeta$  and p-PKM $\zeta$  in a time-dependent manner in the ACC slices. It is noteworthy that protein level of PKM $\zeta$  increases even 5 min after forskolin treatment. This rapid increase of PKM $\zeta$  by elevated cAMP is most likely due to the increase in translation rate, not due to transcriptional regulation [37].

In addition to the cAMP-PKA pathway, other molecules were suggested as upstream of PKM $\zeta$  [4, 9]. Blocking



**Fig. 3** A model for LTP in the ACC. Activation of postsynaptic NMDARs leads to an increase in postsynaptic  $Ca^{2+}$  in dendritic spines.  $Ca^{2+}$  binds to CaM and leads to the activation of calcium-stimulated ACs, primarily AC1. Other  $Ca^{2+}/CaM$ -dependent protein kinases (e.g., PKC, CaMKII, and CaMKIV) are also activated. Activation of CaMKIV, a kinase predominantly expressed in the

nuclei, will trigger CREB signaling pathways. In addition, the activation of AC1 leads to the activation of PKA, and subsequently CREB activation as well. Subsequently, AMPARs likely undergo long-term plastic upregulation. The upregulated PKM $\zeta$  potentiates the response of AMPAR by modulating GluA1-containing AMPARs in the ACC

NMDARs hampers PKM $\zeta$  expression induced by tetanic stimulation [4]. NMDAR signaling especially through an involvement of the GluN2B subunit in the ACC is required for pain processing [60–62]. This implies that the peripheral nerve injury can increase neuronal Ca<sup>2+</sup> level through the activation of NMDARs in the ACC and then, increased Ca<sup>2+</sup> level can induce the PKM $\zeta$  activation. Indeed, elevated Ca<sup>2+</sup> can activate various kinases, including CaMKII, extracellular-signal-regulated kinases, PKA, PKC, PI3-kinase, and mammalian target of rapamycin. These activated kinases can increase the expression of PKM $\zeta$  [9]. Although PKM $\zeta$  expression is controlled by various kinases, newly synthesized PKM $\zeta$  seems to be phosphorylated by PDK1. After strong tetanization of hippocampal slices, PKM $\zeta$  and p-PKM $\zeta$  are simultaneously increased at a similar rate. The ratio of PKM $\zeta$  and p-PKM $\zeta$  does not change even after tetanization [9]. This phenomenon seems to occur in neuropathic pain as well. PKM $\zeta$  and p-PKM $\zeta$  in the ACC increased to similar levels 3 days after nerve injury. However, the expression of PKM $\zeta$  returns to basal level within 7 days after nerve injury, whereas p-PKM $\zeta$  still remains at elevated levels even up to 14 days after nerve injury. Thus, at the initial stage of chronic pain, PKM $\zeta$  might be increased via protein translation and autonomously activated by PDK1. At later stage, expression of PKM $\zeta$  might be suppressed by some homeostatic mechanism. PDK1 still can activate PKM $\zeta$  via a signaling pathway that is unknown so far and thus requires further studies.

### AMPA receptor and PKM $\zeta$

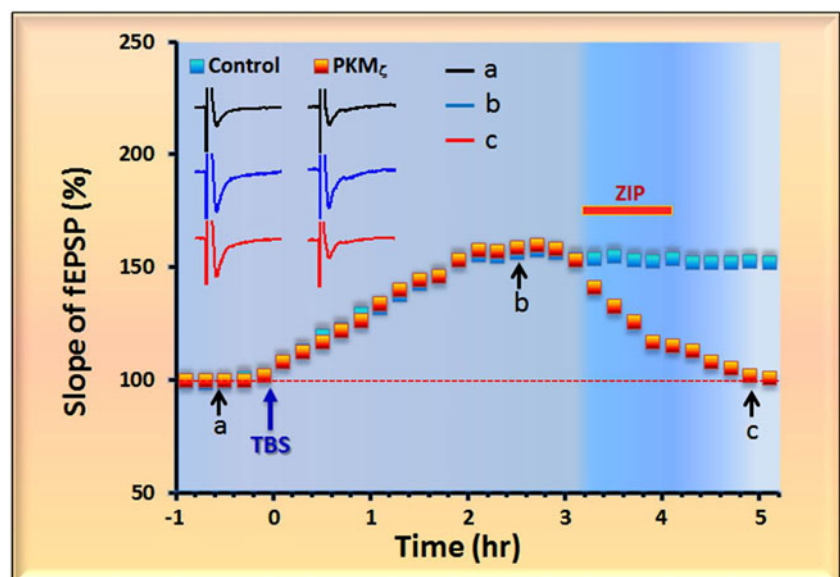
Given the importance of PKM $\zeta$  to the expression of synaptic plasticity, long-term memory and chronic pain,

knowing the downstream effectors of PKM $\zeta$  will help to understand the neuronal mechanism of chronic pain. In the ACC, integrative evidence showed that GluA1 is the downstream target of PKM $\zeta$ . First, deletion of the GluA1 subunit abolished LTP in the ACC, while enhanced LTP was observed in the ACC of GluA2 null mice [29]. Interfering with the interaction between the C terminus of GluA1 and post-synaptic density (PDZ) domain proteins blocked the induction of cingulate LTP [63]. Furthermore, bath application of PhTx-433, an antagonist of Ca<sup>2+</sup>-permeable (i.e., GluA2-lacking) AMPARs, 5 min after LTP induction reduced synaptic potentiation [63]. These data suggest that Ca<sup>2+</sup>-permeable GluA2-lacking receptors contribute to the expression of LTP. Blocking the activities of PKM $\zeta$  by ZIP erased late-phase LTP induced by theta burst stimulation (Fig. 4) [37]. Therefore, PKM $\zeta$  may interact with GluA1 to maintain LTP in the ACC.

Periphery nerve injury resulted in an increase in GluA1 and in its phosphorylation on Ser845, but not GluA2/3, in the ACC neurons [46]. We found that bath application of ZIP decreased the amplitude of evoked EPSCs, and by applying non-stationary fluctuation analysis, we found that the effect of ZIP on the eEPSCs was due to a decrease in the number of active channels, rather than a decrease in the unitary conductance of AMPARs. We further analyzed the subunits of AMPARs and found that blocking the activities of PKM $\zeta$  in the ACC decreased the protein level of GluA1 in the synapses [37]. Therefore, our results suggest that under neuropathic pain condition, PKM $\zeta$  interacts with the GluA1 subunit of AMPARs in the ACC.

The downstream effectors of PKM $\zeta$  were also investigated in other brain areas such as hippocampus [64] and amygdala [15]. In these brain areas, PKM $\zeta$  maintains the L-LTP and long-term memory through persistently modifying

**Fig. 4** ZIP blocked L-LTP in the ACC. Examples showed that TBS can induce L-LTP for at least 5 h in ACC slices of adult mice (blue squares). Bath application of ZIP (5  $\mu$ M) erased the maintenance of L-LTP in the ACC (orange squares). *Inset* Individual traces of field EPSCs are shown at three different time points (before training: *a* L-LTP before ZIP application, *b* after ZIP application, or *c* control; modified from Li et al. [37])



NSF/GluA2-dependent AMPAR trafficking at the synapse [15, 64]. Considering the differences between the hippocampus and the ACC [25], it is possible that the downstream of PKM $\zeta$  may be different between the hippocampus and ACC or they may share at least some of common mechanisms. Future studies need to be done to investigate the contributions of PKM $\zeta$  to the synaptic potentiation in other pain-related brain areas under chronic pain condition.

### Physiological and pathological implications

From the physiological points of view, ACC neurons have multiple dimensions in their functions. They not only respond to sensory stimuli, such as noxious or painful stimuli, but also respond to emotional stimuli. At the single cell level, each cell has a diffuse receptive field that covers almost the whole body area [33, 36]. It is responsive to inputs from somatosensory areas and internal visceral organs. Enhancing excitatory transmission in the ACC after injury may contribute to enhanced attentional state and facilitate individual responses to future potential dangerous stimulation. Recent studies indeed indicate that ACC sends top-down modulatory influences to the spinal cord and facilitates spinal nociceptive responses [50].

ACC influences may not be limited at sensory transmission and modulation. Altered ACC excitability may contribute to emotional anxiety and fear. Recent studies using genetic and pharmacological manipulations demonstrate that ACC neurons contribute to anxiety- and fear-like behaviors [65]. It remains to be determined if ACC produces these effects through other cortical or subcortical structures. It has been known that ACC forms a high density of inter-connections with other brain areas [33].

In summary, cumulative evidence using integrative experimental approaches consistently demonstrate that peripheral injury triggers LTP of excitatory synaptic transmission within the ACC. Enhanced excitatory synaptic transmission may facilitate responses of ACC neurons to subsequent sensory stimulation. In case of chronic pain, ACC LTP may be responsible for increased responses of ACC neurons to peripheral sensory stimuli, including non-noxious stimuli that trigger behavioral allodynia. Potentiated synaptic responses may also contribute to abnormal spontaneous neuronal activity within the ACC, a possible synaptic mechanism for spontaneous pain during chronic pain. Therefore, understanding molecular mechanisms for the induction and expression of ACC LTP may help us to identify new protein targets for the treatment of chronic pain. The discovery of the key role of PKM $\zeta$  in the maintenance of injury-related cortical synaptic potentiation may help us to

design novel medicine such as a selective chemical inhibitor of PKM $\zeta$  for the treatment of chronic pain in patients.

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