Synaptic plasticity in the anterior cingulate cortex in acute and chronic pain

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Abstract | The anterior cingulate cortex (ACC) is activated in both acute and chronic pain. In this Review, we discuss increasing evidence from rodent studies that ACC activation contributes to chronic pain states and describe several forms of synaptic plasticity that may underlie this effect. In particular, one form of long-term potentiation (LTP) in the ACC, which is triggered by the activation of NMDA receptors and expressed by an increase in AMPA-receptor function, sustains the affective component of the pain state. Another form of LTP in the ACC, which is triggered by the activation of kainate receptors and expressed by an increase in glutamate release, may contribute to pain-related anxiety.

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Pain is an unpleasant sensory and emotional experience that serves a vital physiological function in all vertebrates by alerting the organism to the potential for tissue damage. When a harmful stimulus is present, activity in peripheral nociceptors triggers motor reflexes to minimize tissue damage. Acute pain can lead to the formation of a long-lasting fear memory (BOX 1) that allows the organism to link specific environmental signals to an appropriate motor response[1,2]. The expectation of acute pain can also cause anxiety (BOX 2). Whereas fear memory that is associated with acute pain is long-lasting, the unpleasantness associated with it rapidly disappears[1,2]. Unlike acute pain, chronic pain, which is defined in humans as any pain that lasts for several weeks or longer, serves no obvious beneficial function. In addition to being unpleasant, chronic pain is associated with fear (BOX 1), anxiety (BOX 2) and impairment of cognitive function[1–3].

It is known from studies of rodent models that peripheral sensitization of nociceptors and long-term synaptic plasticity in the spinal cord, subcortical areas and cortical areas contribute to chronic pain[3–5]. Although acute and chronic pain states share similar neural pathways, a major challenge is to establish the networks and cellular and molecular mechanisms that differentiate chronic from acute pain. These distinctions are important for the development of drugs that could alleviate chronic pain while minimally affecting acute pain.

Brain-imaging studies in humans have provided crucial information about the cortical regions involved in different types of pain[6,7]. The anterior cingulate cortex (ACC) and other cortical areas, including the insular cortex, primary somatosensory cortex (S1), secondary somatosensory cortex (S2) and prefrontal cortex (PFC), are activated by various acute somatic and visceral noxious stimuli[6–8]. In healthy individuals, the ACC is active during the perception of noxious thermal stimuli. For example, in the thermal grill illusion, non-noxious warm and cold stimuli are arranged in a spatially interlaced pattern that produces an anomalous painful sensation[9]. The ACC is only activated when the warm and cold stimuli are presented together. This strongly suggests that the ACC is involved in signalling the unpleasantness associated with acute pain. Hyperactivation of the ACC has also been consistently noted during functional MRI studies of individuals with neuropathic pain and chronic visceral pain conditions, such as irritable bowel syndrome[6,7]. Interestingly, activation of the ACC has also been associated with emotional or psychological pain — experimentally induced sadness, social exclusion or rejection all lead to an increase in activity in this cortical region[10,11]. It seems likely, therefore, that in chronic pain the ACC contributes to various negative effects, including the unpleasantness of pain.

Cellular and molecular studies using rodent models are beginning to provide insights into the mechanisms that lead to and sustain chronic pain[3–5]. Synaptic plasticity has been reported in many of the structures that are known to be involved in the processing of pain, including the dorsal horn of the spinal cord and a number of cortical structures (BOX 3). Of these higher centres, synaptic plasticity has been most extensively studied in the ACC. In this article, we review the role of the ACC in the processing of acute and chronic pain, based largely on findings from animal models. We then discuss the

Box 1 | **Pain, fear and the anterior cingulate cortex**

In animals, including humans, acute pain can result in the expression of conditioned fear behaviour, which can be triggered by the context or specific stimulus that was associated with the painful episode. Chronic pain, by contrast, is associated with fear of aggravating the pain (usually referred to in the human literature as 'fear avoidance'). Cumulative evidence suggests that anterior cingulate cortex (ACC) neurons are actively involved in fear memories.

Classical fear conditioning in rodents activates neurons in the ACC, in addition to the amygdala and hippocampus[69]. Furthermore, electrical activation of ACC neurons causes freezing in freely moving animals and creates a conditioned fear memory for the same location[115]. Similarly, chemical activation of ACC neurons in rodents induces aversion to the place where the activation occurred[43]. Conversely, bilateral (but not unilateral) chemical inactivation of the ACC inhibited the formation of conditioned fear memory induced by footshock[115,119,120]. Bilateral microinjection of NMDA-receptor antagonists into the ACC also reduced or blocked the formation of a fear memory[57].

Taken together, these results suggest that ACC neurons are involved in fear learning. In addition, recent studies in rodents show that the firing pattern of ACC neurons is altered during trace fear conditioning[73,121], and these changes may be predictive of the aversive event. In humans, as in rodents, trace fear conditioning activates several brain areas, including the ACC[122].

The ACC also has a critical role in fear memory for remote events. In experiments using contextual fear conditioning, it was found that the retrieval of a remote memory (36 days after conditioning), but not of a recent contextual fear memory (1 day after conditioning), increased the expression of early growth response 1 (*Egr1*) in the ACC, and that lidocaine infusion into the ACC disrupted the remote fear memory[123]. Observational fear learning, whereby an animal acquires fear learning by observing the behaviour of a conventionally trained animal, has also been reported to require ACC activity[120].

Although it is known that acute nociception can trigger fear-memory formation[25,26], the relationship between fear memory and chronic pain has not been as extensively investigated. However, in humans with chronic pain, fear is one of the key factors that contribute to suffering[124–126]. In rodents, trace fear conditioning triggers potentiation of AMPA-receptor-mediated responses in the ACC[121], and this also occurs in models of chronic pain[99]. Furthermore, in rodent models of chronic pain, it has been demonstrated that the formation of novel trace fear memories is consistently impaired[100]. The synaptic changes that occur in the ACC in rodent models of chronic pain may explain, at least in part, why humans with chronic pain show impaired cognitive function[100].

different forms of synaptic plasticity that have been identified in the ACC and the implications of these forms of plasticity for pain processing.

The ACC within the nociceptive pathway

Anatomy. The ACC forms the frontal part of the cingulate cortex[12] and consists of layers I, II–III and V–VI in both rodents and humans[12,13]; unlike other cortical regions, the ACC lacks a layer IV. Layer I contains interneurons and projection fibres from other CNS regions; some of these form connections with neurons in the deeper layers of the ACC. Layers II–III contain predominantly pyramidal cells. The deeper layers of the ACC contain pyramidal cells and interneurons[12,13], and many of these send their projections to other cortical areas, such as the insular cortex, and to subcortical areas and the spinal cord[12–14] (FIG. 1). The dendrites of deep-layer pyramidal cells spread into the superficial layers of the ACC, and so it is likely that neurons between different layers of ACC also form synaptic connections. However, few studies have examined functional connections between different layers in the ACC, although some recent studies using dual whole-cell patch-clamp recording have provided some insights. Different types of monosynaptic connections, including pyramidal neuron–pyramidal neuron, inhibitory neuron–pyramidal neuron and inhibitory neuron–inhibitory neuron, have been identified[15]. Some of these connections are reciprocal, suggesting that the ACC has a complex network architecture[15].

Nociceptive information from somatic and visceral organs is conveyed indirectly to the ACC through at least three major projection systems. The first of these is from the thalamus; ACC neurons receive nociceptive sensory inputs from the medial thalamus[16,17], which in turn receives input from spinal projection fibres through the spinothalamic tract. Electrophysiological experiments using *in vivo* thalamic stimulation have provided functional confirmation of these projections[18,19]. Recently, it was also shown that mediodorsal thalamic neurons directly excite parvalbumin-positive interneurons in the dorsal ACC, which are thought to mediate feedforward inhibition of pyramidal neurons in layers II–III[20]. Nociceptive information is also conveyed to the ACC through the amygdala, including the central nucleus, which in turn receives spinal sensory input through the parabrachial area[21,22]. The third source of nociceptive input to the ACC is from other pain-related areas of the cortex, such as S1 (REF. 23) and the insular cortex (FIG. 1).

Pyramidal cells in layer V project to subcortical structures such as the hypothalamus and periaqueductal grey[24], the latter of which is involved in the descending modulation of spinal sensory transmission (FIG. 1). Furthermore, a direct projection from the deep pyramidal cells of the ACC to the spinal cord, including the dorsal horn, has been reported[14]. ACC neurons also form connections with neurons in the amygdala, a structure that has a key role in processing fear and anxiety[25,26]. In addition, stimulation of the ACC in various mammalian species can induce vocalization[13] that is thought to be an indicator of pain or fear, presumably through a projection to the motor cortex. ACC neurons also project to the PFC[27], and such connections may influence sleep and cognition. A projection from the ACC to the locus coeruleus, a region that influences thermal pain thresholds in mice, has also been described[28,29]. Furthermore, the ACC is innervated by dopaminergic, serotonergic, cholinergic and adrenergic fibres that originate in subcortical regions[30]. Although little is known about the specific roles of these projections, it may be presumed that this widespread connectivity contributes to the complex role of the ACC in processing pain and its associated emotions.

Physiology. Fast, excitatory responses in the ACC, evoked experimentally by low-frequency stimulation, are predominantly mediated by AMPA receptors (AMPARs)[31]. However, kainate receptors can also contribute to fast, excitatory synaptic transmission in pyramidal neurons. Kainate-receptor-mediated excitatory postsynaptic currents (EPSCs) have slower kinetics than do AMPAR-mediated EPSCs, summate effectively during high-frequency transmission and involve activation of both GluK1 (also known as GluR5) and GluK2 (also known as GluR6) subunits[31]. In the ACC, NMDA receptors (NMDARs) mediate even slower excitatory responses

Acute pain
Pain that is associated with a noxious stimulus and that does not persist when the noxious stimulus is removed.

Fear memory
A type of associative memory in which a fear response is triggered by a context or a neutral stimulus that was previously associated with an aversive event.

Anxiety
An affective state reflecting a feeling of unease, worry or fear.

Chronic pain
Long-lasting pain that is associated with a chronic disease, or an aberrant type of pain that persists beyond recovery from disease or injury.

Box 2 | **Pain, anxiety and the anterior cingulate cortex**

In humans, it is known that anxiety, as well as fear, can enhance pain perception and that chronic pain can lead to persistent anxiety[124–126]. Furthermore, anxious individuals are more likely to develop chronic pain[125], and anxiolytic drugs can reduce chronic pain in some patients[124,125]. Increased activity in the anterior cingulate cortex (ACC) has been reported in individuals who are exposed to anxiety-inducing settings[127] and in individuals with anxiety disorders[128]. Thus, chronic pain and anxiety may be mutually reinforcing.

Similar conclusions have been reached in rodent experiments. Anxiety-like behaviours are significantly increased in rodent models of chronic pain compared with controls[59,129]. Conversely, high levels of baseline anxiety in rodents contribute to increased acute visceral nociceptive responses[130]. The anatomical connections of the ACC with the amygdala and other subcortical regions involved in emotional responses[131–133] provide a rationale for a potential role for the ACC in the processing of anxiety and fear in relation to painful stimuli or experiences. Permanent lesion or chemical inactivation of the ACC in rodents also produces anxiolytic effects[134]. Furthermore, a recent study found that selective optogenetic activation of pyramidal neurons of the ACC in mice induced anxiety- and depression-like behaviour[48]. These findings further support the idea that activity in ACC neurons influences anxiety.

In a mouse model of chronic gastrointestinal pain, microinjection of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker ZD7288 into the ACC reduced anxiety-like behaviour[59,110], suggesting that disruption of synaptic plasticity in this region can interfere with the interaction between pain and anxiety.

Presynaptic long-term potentiation (LTP) is a possible synaptic mechanism in the ACC that may contribute to anxiety-like behaviour in the context of chronic pain[59]. For example, high levels of anxiety prevent the induction of presynaptic LTP in the ACC. In addition, application of ZD7288, which is anxiolytic on injection into the ACC, reverses established presynaptic LTP. It also specifically inhibits synaptic transmission in ACC neurons that are active in mouse models of neuropathic pain[59]. In summary, ACC neurons participating in the response to chronic pain receive potentiated thalamocortical inputs owing to presynaptic LTP, and this signal may contribute to the associated anxiety.

It is known that presynaptic LTP and postsynaptic LTP can occur together in ACC neurons[59], and thus the additive effects of these two forms of LTP could provide a substrate for the mutually reinforcing effects of chronic pain and anxiety. Specifically, the unpleasantness of chronic pain may be encoded by postsynaptic LTP and involve alterations in AMPA-receptor function, whereas the anxiety associated with chronic pain may be encoded by presynaptic LTP, which involves an increase in the probability of transmitter release. As the signal (unpleasantness) and salience (the level of anxiety associated with the unpleasantness) are encoded by entirely independent mechanisms, they may be additive, even at the level of an individual synapse. In principle, a synapse in which postsynaptic LTP is saturated could still be further potentiated by the induction of presynaptic LTP. However, whether the relationship between pain and anxiety in the ACC occurs at the level of the synapse or the level of the neuron is not yet known.

Neuropathic pain
A type of chronic pain caused by a lesion or disease of the peripheral nervous system or the CNS.

Fear conditioning
A behavioural task in which an animal learns to associate a neutral stimulus (for example, a tone) with an aversive event (for example, a footshock). See the glossary definition for 'fear memory'.

Trace fear conditioning
A form of fear conditioning in which a time interval is interposed between the conditioned stimulus and the unconditioned stimulus.

that also summate effectively during high-frequency transmission[32,33]. Finally, metabotropic glutamate receptors (mGluRs) can modulate synaptic transmission in the ACC[34]. As elsewhere in the cortex, GABA is the major inhibitory transmitter in the ACC, where it acts on both type A GABA ($GABA_A$) receptors[35] and $GABA_B$ receptors[36,37]. Activation of GluK1-containing kainate receptors in the ACC can regulate GABA release[35].

Defining the role of ACC in pain

Neurons in the ACC respond to both noxious and non-noxious mechanical or thermal somatosensory stimuli[38–41]. In particular, pyramidal neurons, which integrate inputs from the various cortical layers, are excited by noxious mechanical inputs[38,41]. Non-pyramidal cells in the ACC are inhibitory neurons that contain GABA and various neuropeptides[42]. In the local circuits, inhibitory neurons receive excitatory glutamatergic innervation from pyramidal cells, resulting in feedback release of GABA on to the perisomatic region of pyramidal cells. Recordings from the ACC, in both humans and experimental animals, show that many ACC pyramidal neurons have diffuse receptive fields[39,40].

In several rodent models of chronic pain, chemical or electrolytic lesions of the ACC attenuate the affective component of the pain state, which is reflected in reduced conditioned place aversion induced by a noxious stimulus[43–46] and similarly by a reduction in place escape or avoidance[47]. Conversely, in naive rodents, electrical stimulation of the ACC in the absence of any peripheral noxious stimulation induces aversion to the place where it was delivered, indicating that activation of this region is sufficient to induce behaviour consistent with the affective component of pain[43]. Notably, however, in rodent models of neuropathic pain, mechanical allodynia is not consistently attenuated by lesions of the ACC, suggesting that other cortical areas might also be involved in the sensory components of this chronic pain state[45,48,49].

Optogenetic studies have shown that specific activation of pyramidal neurons in the ACC lowers mechanical pain thresholds in mice[42]. However, when the same procedure was carried out in a mouse model of inflammatory pain induced by subcutaneous injection of complete Freund's adjuvant (CFA) to the hindpaw, in which the mice already had lowered mechanical nociceptive thresholds, optogenetic stimulation of the pyramidal neurons of the ACC did not further lower the mechanical threshold. This occlusion between the effects of optogenetic stimulation and CFA treatment suggests that activation of ACC pyramidal neurons is sufficient to mediate hypersensitivity. Furthermore, it has been shown that optogenetic stimulation of inhibitory GABAergic interneurons in the ACC has an inhibitory effect on nociception. One study[50] reported that nociceptive behaviours induced by subcutaneous injection of formalin into the hindpaw were attenuated by optogenetic activation of the ACC in mice in which channelrhodopsin 2 was expressed predominantly in inhibitory interneurons under the control of the thymus cell antigen 1, theta (*Thy1*) promoter. In a second study, in which a Cre–*loxP* approach allowed more-accurate cell type-specific expression of channelrhodopsin 2, it was also shown that optogenetic activation of parvalbumin-positive interneurons, but not somatostatin-positive interneurons, reduced CFA-induced mechanical hypersensitivity[42]. This inhibitory effect on mechanical hypersensitivity was likely to have been mediated by inhibition of pyramidal neurons in the ACC, because the effect on nociception was comparable to that induced by optogenetic inhibition of firing in calcium/calmodulin-dependent protein kinase type II (CaMKII)-expressing pyramidal cells. In other words, activation of ACC pyramidal neurons is also necessary for the mediation of hypersensitivity. In summary, the recent application of optogenetics to the study of pain is allowing dissection of the effects of acute manipulation of the role of specific neuronal types in the sensory, affective and cognitive components of pain.

Box 3 | **Pain-associated synaptic plasticity in other CNS regions**

Dorsal horn of the spinal cord
Sensory synapses, including those of C fibres carrying pain-related information from the periphery to the dorsal horn, can undergo long-term potentiation (LTP)[135]. Synaptic transmission between primary afferent fibres and spinal dorsal horn neurons is significantly potentiated in animal models of neuropathic pain[136] and inflammatory pain[137], as shown *in vivo* or in slice recordings. Low-frequency (1 Hz) stimulation of C fibres induces LTP in lamina I cells that project to the parabrachial area[137].

Thalamus
Although enhanced firing rates in some neurons in the thalamus have been reported in rodent models of neuropathic pain[138,139] and inflammatory pain[140], it has not yet been determined whether LTP or long-term depression (LTD) occurs in the connection between spinothalamic projection neurons and thalamic neurons.

Amygdala
Excitatory synapses between inputs from the parabrachial area to the central nucleus of amygdala are enhanced in animal models of inflammatory pain[141] and neuropathic pain[142,143].

Insular cortex
In brain-slice preparations, excitatory synapses in the insular cortex can undergo LTD[144] or LTP[145]. Excitatory transmission, including NMDA-receptor- and AMPA-receptor-mediated responses[106,146], is enhanced here in models of neuropathic pain.

Prefrontal cortex
Excitatory transmission in the prefrontal cortex is enhanced in rodent models of neuropathic pain[105].

Somatosensory cortices (S1 and S2)
Excitatory transmission in the primary somatosensory cortex (S1) and secondary somatosensory cortex (S2) is enhanced in models of neuropathic pain, and this may contribute to increased excitation of anterior cingulate cortex (ACC) neurons through the S1 and S2 to ACC pathways[23].

Synaptic plasticity in the ACC

Long-term potentiation (LTP) and long-term depression (LTD) are forms of synaptic plasticity that have been widely studied in the context of learning and memory[51,52]. Chronic pain can be thought of as a type of persistent sensory memory, and increasing evidence suggests that LTP and LTD in the dorsal horn of the spinal cord and cortical areas, including the ACC[3,4], are causally related to chronic pain. There is abundant evidence from animal models to suggest that, in the spinal cord, LTP at C fibre synapses can contribute to hyperalgesia, and that reversal of LTP by depotentiation reduces nociceptive behaviours[53]. Here, we describe the forms of synaptic plasticity that can be induced in the ACC *in vitro*.

Induction of long-term potentiation. LTP can be readily monitored using various experimental methods, including field excitatory postsynaptic potential (EPSP) recordings, whole-cell patch-clamp recordings and multi-electrode array recordings. Using field recordings in slices from adult mice, it has been shown that, in response to theta-burst stimulation, glutamatergic synapses in the ACC exhibit LTP lasting many hours[54,55]. Induction of this form of LTP requires the activation of NMDARs and L-type voltage-gated calcium channels (L-VGCCs)[56]. LTP can also be induced in the ACC using a number of other protocols, including stimulus–depolarization pairing and spike–EPSP pairing[57], as detected by whole-cell patch-clamp recording. LTP induced by such pairing protocols is typically triggered by the activation of NMDARs but does not require the activation of L-VGCCs[57].

There are various subtypes of NMDAR, each of which is composed of different combinations of subunits: typically, two GluN1 (also known as NR1) subunits and two GluN2 (also known as NR2) subunits, of which there are four possible subtypes, GluN2A–D (also known as NR2A–D). Although many studies have investigated which NMDAR subunit composition is required to trigger LTP in hippocampal area CA1, there is still some controversy regarding the relative role of the GluN2A- and GluN2B-containing NMDAR subtypes. Indeed, recent work suggests a predominant role for NMDAR tri-heteromers, which consist of GluN2A and GluN2B (together with the obligatory pair of GluN1 subunits), in the induction of LTP in area CA1 of adult tissue[58]. In the ACC, it has been shown that LTP, as detected by whole-cell patch-clamp recording, is sensitive to both GluN2A-preferring and GluN2B-preferring antagonists[57], suggesting that tri-heteromers of the NMDAR may also be the dominant form of the receptor that contributes to LTP at these synapses.

An NMDAR-independent form of LTP can also be readily induced in the ACC by paired-pulse low-frequency stimulation[59]. This form of LTP cannot be induced in ACC slices obtained from mice lacking the GluK1 subunit, but can be induced in slices from mice lacking the GluK2 subunit, and is blocked by a potent GluK1-selective kainate receptor antagonist, UBP310 (REF. 60). LTP that is dependent on GluK1-containing kainate receptors closely resembles a form of NMDAR-independent LTP that has been described at mossy fibre synapses in the hippocampus[60,61], as well as at thalamic inputs to the lateral amygdala[62].

Transduction of long-term potentiation. At hippocampal synapses, the activation of NMDARs leads to an increase in Ca^{2+} levels in dendritic spines, owing to Ca^{2+} entry through NMDARs and a consequent Ca^{2+}-stimulated release of Ca^{2+} from intracellular stores[63]. The postsynaptic Ca^{2+} signal is an essential component for the induction of LTP in rat hippocampal slices[64]. Similar mechanisms operate in the ACC, as postsynaptic chelation of Ca^{2+} blocks the induction of LTP in mouse ACC slices[57]. Electroporation of a mutant form of calmodulin (CaM) with disrupted calcium-binding sites also prevents the induction of LTP in the ACC[65], suggesting that the activation of CaM-dependent signalling pathways by Ca^{2+} binding is essential.

Adenylyl cyclase 1 (AC1) and AC8, two adenylyl cyclase subtypes that are activated by Ca^{2+}–CaM[66], are both present in the ACC. AC1 is highly expressed in most cortical layers[67], and mice lacking AC1, but not AC8, fail to show LTP[56]. Furthermore, pharmacological inhibition of AC1 by a selective inhibitor, NB001, abolishes LTP induction in mouse ACC neurons *in vitro*[68]. As with LTP in the hippocampus, it seems likely that AC1 generates cyclic AMP, which activates protein kinase A (PKA), and that this is required for LTP transduction in the ACC, although this has yet to be confirmed. Another

Affective component of the pain state
The feeling of unpleasantness that is associated with pain.

Allodynia
An abnormal type of pain that is caused by a stimulus that typically does not evoke pain.

Hyperalgesia
A condition in which the level of pain arising from a particular painful stimulus is greater than would normally arise from that stimulus.

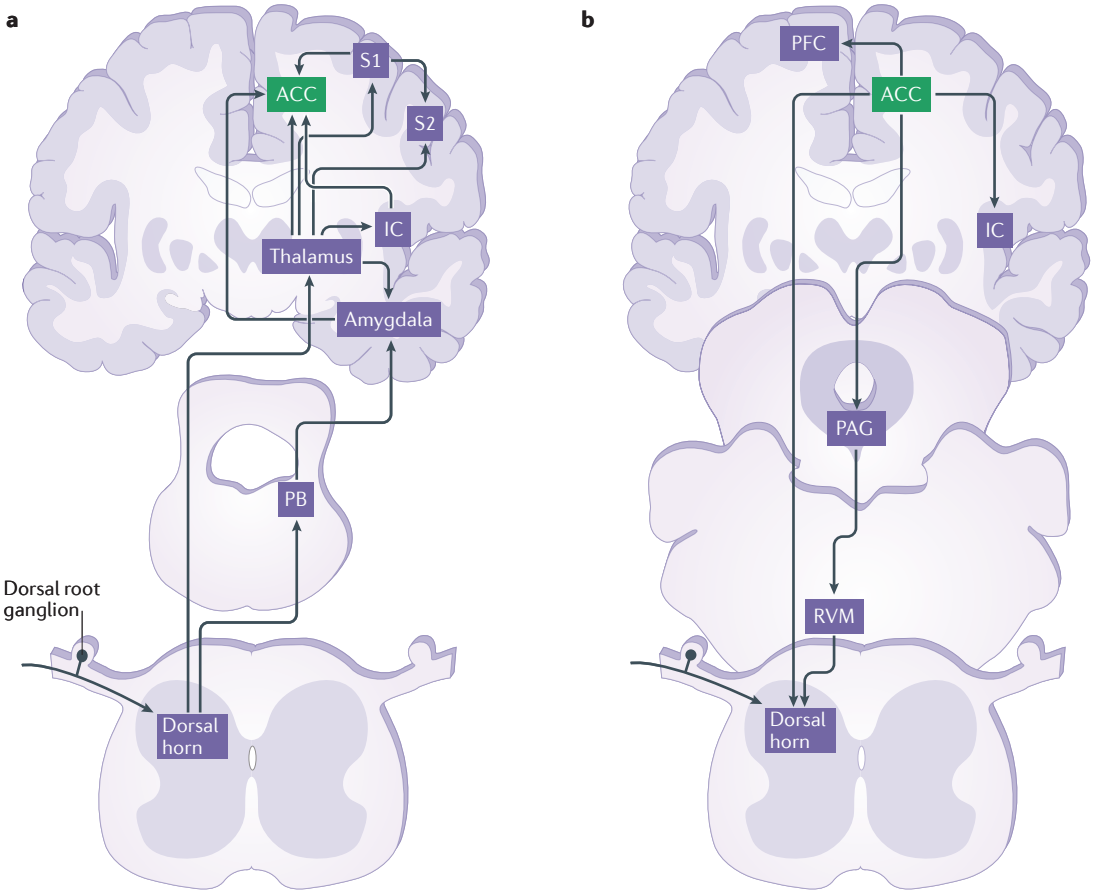

Figure 1 | **Major sensory inputs to and outputs from the ACC. a** | Anterior cingulate cortex (ACC) neurons receive inputs from various cortical and subcortical structures. These inputs convey nociceptive information through the thalamus, as well as information from CNS regions involved in the mediation of emotional states, such as the amygdala and insular cortex (IC). **b** | ACC neurons project to CNS regions that mediate sensory modulation, anxiety, fear and memory. Neurons in the deep layers of the ACC also send their projections directly or indirectly to the dorsal horn of the spinal cord. This top-down circuit allows cortical neurons to directly modulate the sensory input into the CNS. Descending facilitation of sensory inputs may increase the sensitivity of cortical neurons to peripheral sensory stimuli under physiological conditions, but tonic facilitation may also contribute to enhanced pain in chronic pain conditions. Together, this positive spinal dorsal horn–thalamus–cortex–spinal dorsal horn loop may also provide a circuit for central sensitization. Note that most projections are bilateral, but for simplicity only one side is shown here. PAG, periaqueductal grey; PB, parabrachial area; PFC, prefrontal cortex; RVM, rostroventral medulla; S1, primary somatosensory cortex; S2, secondary somatosensory cortex.

CaM-sensitive protein, CaMKIV, is enriched in ACC synapses[69] and is required for the induction of LTP in the ACC[69], where it may function to activate the transcription factor cAMP response element (CRE)-binding protein (CREB). The zinc finger transcription factor early growth response protein 1 (EGR1; also known as NGFIA, KROX24 and ZIF268) is crucial for coupling extracellular signals to changes in cellular gene expression and has been shown to be important for synaptic plasticity in the hippocampus[70]. The upstream promoter region of the *Egr1* gene contains CREs, binding sites for CREB, which suggests that EGR1 might act downstream from the CREB pathway. Consistent with this, in mice lacking *Egr1*, only a short-lasting form of LTP can be induced in the ACC[71]. Similarly, analysis of mice with a functional deletion of *Fmr1*, which encodes fragile X mental retardation protein (FMRP) — a translational suppressor that affects the synthesis of plasticity-related molecules such as activity-regulated cytoskeleton-associated protein (ARC), αCaMKII and microtubule-associated protein 1B (MAP1B)[72] — has also indicated that protein synthesis is required for the full expression of LTP in the ACC[73]. Extracellular signal-regulated kinases (ERKs) have also been shown to be necessary for the induction of LTP in the ACC[74].

The transduction of the kainate-receptor-dependent form of LTP in the ACC also involves activation of the cAMP signalling pathway (FIG. 2). Furthermore, activation of AC1, but not AC8, is required for the transduction of kainate-receptor-dependent LTP in the ACC[59], which distinguishes this form of LTP in the ACC from LTP at mossy fibre synapses in the hippocampus, where both AC1 and AC8 are required[75]. Kainate-receptor-dependent LTP in the ACC may also involve FMRP signalling[76].

Central sensitization
Increased responsiveness of nociceptive neurons in the CNS to their normal or subthreshold afferent input.

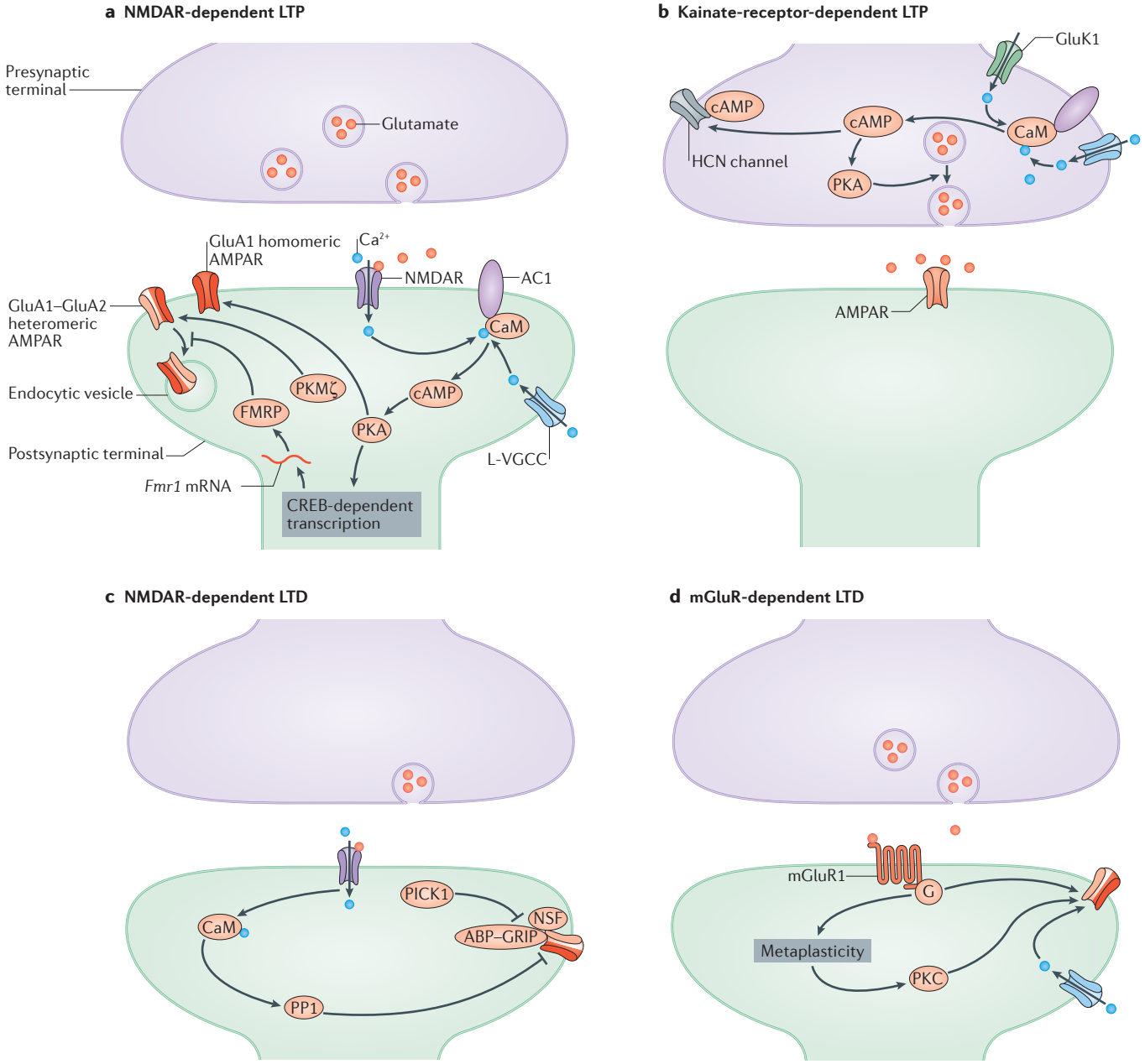

Figure 2 | **Forms of long-term potentiation and long-term depression in the ACC. a**,**b** | Two forms of long-term potentiation (LTP) can be recorded in the anterior cingulate cortex (ACC): postsynaptic LTP and presynaptic LTP. The two forms may occur at the same synapses. Induction of postsynaptic LTP (part **a**) requires the activation of NMDA receptors (NMDARs; this is referred to as NMDAR-dependent LTP in the text), and the maintenance of postsynaptic LTP expression requires the postsynaptic activity of an atypical protein kinase C (PKC) isoform (most probably PKMζ). Adenylyl cyclase 1 (AC1) is also crucial for the induction of postsynaptic LTP in the ACC. Activation of cyclic AMP-dependent protein kinase (PKA) drives the insertion of Ca^{2+}-permeable AMPA receptors (CP-AMPARs; GluA1 homomers). PKA also translocates to the nucleus, where it phosphorylates the transcription factor cAMP response element-binding protein (CREB), leading to the synthesis of several downstream plasticity proteins, including fragile X mental retardation protein (FMRP; encoded by *Fmr1*). PKMζ may maintain LTP by upregulating GluA1–GluA2 heteromers. In addition, increased levels of FMRP may enhance the postsynaptic function of AMPARs as well as NMDARs. Presynaptic LTP (part **b**) in the ACC is not dependent on NMDARs; instead, presynaptic kainate receptors and AC1 activation are necessary for its induction, and the expression of presynaptic LTP may require the activity of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. cAMP binds to the HCN channel to increase its sensitivity, and PKA enhances vesicle fusion. **c**,**d** | Long-term depression (LTD) can also be induced at ACC synapses, and can be separated into at least two forms: NMDAR- and metabotropic glutamate receptor (mGluR)-dependent LTD. Both forms of LTD require postsynaptic modification of AMPARs for their expression. For NMDAR-dependent LTD (part **c**), calmodulin (CaM) binds to Ca^{2+} that enters through NMDARs, and this activates protein phosphatase 1 (PP1). PP1 may dephosphorylate AMPARs to effect the reduced response. In addition, protein interacting with C kinase 1 (PICK1) may affect the interaction between GluA2 and AMPAR-binding protein–glutamate receptor-interacting protein (ABP–GRIP). mGluR-dependent LTD (part **d**) involves activation of mGluR1 with an additional contribution from L-type voltage-gated calcium channels (L-VGCCs), and is regulated by a PKC-dependent form of metaplasticity. NSF, *N*-ethylmaleimide-sensitive factor.

Expression of long-term potentiation. Evidence from genetic and pharmacological studies indicates that changes in postsynaptic AMPARs contribute to the expression of NMDAR-dependent LTP in the ACC. AMPARs are heteromeric assemblies of different subunits. Of these, the GluA1 (also known as GluR1) subunit seems to have a crucial role in the expression of LTP in the hippocampus[77,78]. Similarly, the GluA1 subunit is crucial for expression of LTP in the ACC. For example, intra-ACC administration of a peptide that mimics the PDZ domain at the carboxy-terminal tail of the GluA1 subunit blocks the early expression of LTP, whereas peptides that interfere with interactions with the C-terminal tail of GluA2 (also known as GluR2) or GluA3 (also known as GluR3) do not[79]. Furthermore, LTP in the ACC is readily induced in mice lacking GluA2, whereas in mice lacking GluA1 it is absent[80]. In the hippocampus, LTP can involve the transient insertion of GluA2-lacking, and therefore Ca^{2+}-permeable, AMPARs (CP-AMPARs)[81]. Similarly, at ACC synapses, the application of a CP-AMPAR antagonist 5 min after induction of LTP reduced synaptic potentiation[79]. Recent evidence suggests that CP-AMPARs are specifically associated with the PKA-dependent form of LTP at CA1 synapses[82]. This component of CA1 LTP is therefore very similar to the LTP described at ACC synapses.

The GluA2 subunit may also be required for the full expression of LTP. For example, at CA1 synapses, there is evidence that protein kinase Mζ (PKMζ) is required to maintain persistent synaptic potentiation[83] by stabilizing the interaction between the GluA2 subunit and the vesicular-fusion protein *N*-ethylmaleimide-sensitive factor (NSF)[84]. As in the hippocampus, administration of an inhibitor of PKMζ, zeta inhibitory peptide (ZIP), abolished LTP in the ACC[54]. Recent studies have questioned the selectivity of this peptide for PKMζ[85,86] (but see REF. 87) and, indeed, it is likely that ZIP may also inhibit the other atypical protein kinase C (PKC) isoform, PKCι/λ[83,87]. Future studies are therefore required to establish the precise mechanism underlying the inhibition of LTP in the ACC by administration of this peptide.

In contrast to NMDAR-dependent LTP, kainate-receptor-dependent LTP is expressed by an increase in the probability of release, *P*(r), as assessed by changes in paired-pulse facilitation. In the hippocampus, it has been claimed by one laboratory[88] but disputed by another[89] that the expression of mossy fibre LTP involves the modulation of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, leading to a persistent depolarization of presynaptic terminals — an effect that could account for the increase in *P*(r). In the ACC, a similar mechanism has been suggested, based on sensitivity of this form of LTP to an HCN channel inhibitor, ZD7288 (REF. 59). There are four HCN channel subunits (HCN1–4), all of which are expressed in both the ACC and the thalamus. Further work is required to identify the molecular target of ZD7288 that is required for the maintenance of kainate-receptor-dependent LTP.

Long-term depression. Two major forms of LTD have been characterized in the hippocampus; one form is triggered by the activation of NMDARs, and the other is triggered by the activation of mGluRs[90]. Both forms of LTD have also been observed in the rodent ACC[91–93] (FIG. 2). Low-frequency stimulation (1–5 Hz) for a prolonged period (3–15 min) induces input-specific LTD in the ACC that requires the activation of mGluR1 and L-VGCCs[91,92], whereas antagonism of NMDARs has only a minor effect on the induction of this form of LTD[92]. However, an NMDAR-dependent form of LTD in the ACC has also been observed in studies that used a pairing protocol[93,94]; this form of LTD is sensitive to both GluN2A- and GluN2B-preferring antagonists and requires an increase in postsynaptic Ca^{2+} and CaM levels[65,93]. By analogy with the hippocampus, this may trigger a protein phosphatase cascade involving calcineurin and protein phosphatase 1 (PP1)[90].

NMDAR-dependent LTD seems to involve an alteration in the function of AMPARs, particularly through the C-terminal tail of the GluA2 subunit. Thus, in the ACC, LTD is abolished in mice lacking GluA2 but is intact in mice lacking GluA3 (REF. 94), and it is blocked by administration of an exogenous peptide that prevents interactions between the PDZ domain at the C terminus of GluA2 and one or more of its interaction partners (for example, glutamate receptor-interacting protein (GRIP) or protein interacting with C kinase 1 (PICK1)). NMDAR-dependent LTD is also blocked by another exogenous peptide that interferes with the interaction between GluA2 and NSF, suggesting that displacement of NSF by the clathrin–adaptor protein 2 complex is also involved in NMDAR-dependent LTD in the ACC[94] (FIG. 2).

To summarize, the studies carried out to date suggest that LTD in the ACC shares similar mechanisms with LTD in the hippocampus. This reinforces the view that a relatively small subset of generic synaptic plasticity mechanisms is used throughout the brain, where these mechanisms have a large variety of region-specific functions.

Findings from chronic pain models

Long-term potentiation and long-term depression. In a rodent model of neuropathic pain, synaptic responses in the ACC are potentiated at the time that allodynia develops, typically 1–2 weeks after nerve injury[54,59]. At this time, too, the late component of LTP can no longer be induced by theta-burst stimulation, indicating that maximal potentiation has already occurred. These findings suggest that neuropathic pain is linked to the mechanisms that underlie the expression of LTP in the ACC.

Expression of activity-dependent immediate early genes, such as *Fos* and *Egr1*, is linked to the induction of late LTP in the hippocampus, and their expression is commonly used as a marker for LTP in studies of learning and memory[64]. Consistent with the idea that chronic pain is linked to persistent LTP in the ACC, rodent models of chronic inflammatory pain, neuropathic pain, bone-cancer pain and chronic visceral pain are all associated with the activation of *Fos* and *Egr1* in ACC neurons[14,91,95]. In addition, there is increased neuronal

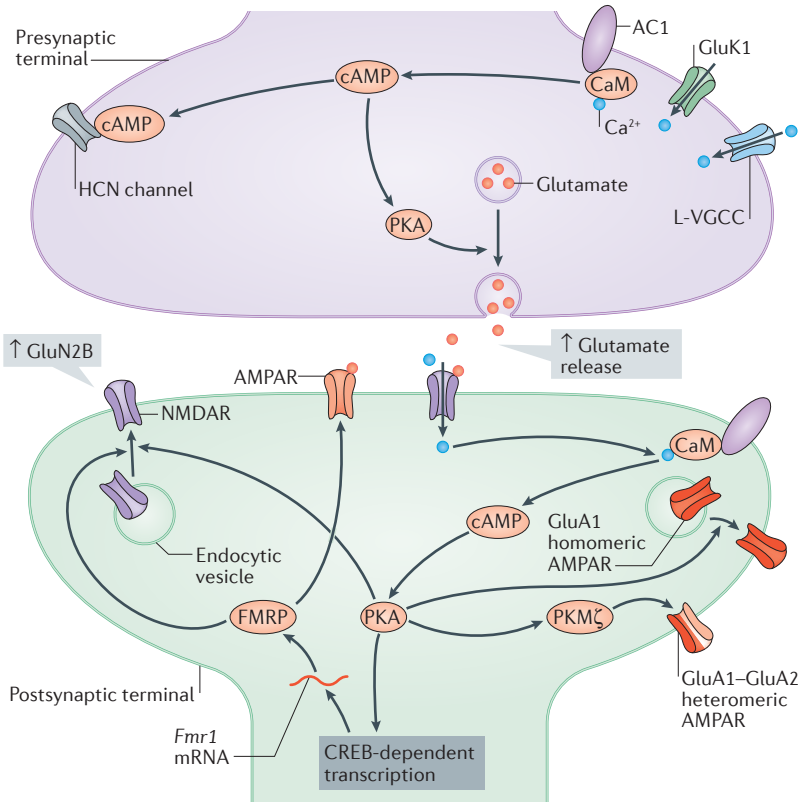

Figure 3 | **Signalling pathways that mediate the upregulation of excitatory transmission in the ACC in rodent models of chronic pain.** Synapses in the anterior cingulate cortex undergo long-term presynaptic and postsynaptic changes. At presynaptic sites, glutamate release is increased; at postsynaptic sites, the expression of AMPA receptors (AMPARs) is increased. These presynaptic and postsynaptic changes that occur in chronic pain models share some common mechanisms with presynaptic long-term potentiation (LTP) and postsynaptic LTP that can be induced by experimental protocols *in vitro* (FIG. 2). In addition to postsynaptic increases in AMPAR expression, expression of postsynaptic NMDA receptors (NMDARs), particularly those containing the GluN2B subunit, is increased in models of chronic pain. Adenylyl cyclase 1 (AC1) is essential for the presynaptic enhancement of glutamate release and the postsynaptic potentiation of AMPARs and NMDARs. CaM, calmodulin; cAMP, cyclic AMP; CREB, cAMP response element-binding protein; FMRP, fragile X mental retardation protein (encoded by *Fmr1*); HCN, hyperpolarization-activated cyclic nucleotide-gated; L-VGCC, L-type voltage-gated calcium channel; PKA, protein kinase A; PKMζ, protein kinase Mζ.

excitability[96] and evidence for synaptic potentiation in the ACC in models of chronic pain. For example, peripheral-digit amputation, an animal model for phantom pain, produces a long-lasting enhancement of synaptic responses in the ACC to either local or peripheral stimulation *in vivo*[97]. Peripheral nerve-block experiments have demonstrated that peripheral nerve activity is not required for the maintenance of amputation-induced potentiation of synaptic responses evoked in the ACC, indicating that synaptic plasticity in the ACC can be maintained by central mechanisms[97].

Pain-related long-term changes in synaptic transmission are not limited to potentiation. LTD induced by repetitive stimulation of ACC neurons is also strikingly affected after peripheral tissue injury. For example, in rodents with digit or tail-tip amputation, the induction of LTD in the ACC, measured 45 min and 2 weeks after amputation, is suppressed[91,92]. Furthermore, in a mouse model of bone-cancer pain, LTD in the ACC is impaired[98] (FIG. 3). On the basis of these observations, we propose that chronic pain is associated with a saturated late component of LTP, and with a suppression of LTD, in the ACC.

AMPA receptors. AMPAR-dependent EPSCs that can be recorded from pyramidal neurons of layers II–III and V, and evoked by focal electrical stimulation within the ACC, are increased after peripheral nerve ligation in mice[14,99] (FIG. 3). Similar changes are found in ACC neurons in a murine model of inflammation induced by CFA[100,101]. These changes are associated with an alteration in the rectification of AMPAR-mediated synaptic transmission and the development of sensitivity to an inhibitor of CP-AMPARs[14,102]. Consistent with this, the population of membrane-bound GluA1-containing AMPARs is increased, whereas GluA2- and/or GluA3-containing AMPARs are not significantly affected. The selective contribution of GluA1 to the increase in EPSCs is further supported by genetic studies. Deletion of GluA1, but not GluA2, significantly reduces peripheral injury-triggered *Fos* activation in the ACC, as well as in the spinal cord dorsal horn, and behavioural responses to the peripheral injury are also reduced[103]. Recent electron microscopy data further support the conclusion that postsynaptic GluA1-containing receptors are increased after peripheral injury[102].

NMDA receptors. In addition to the enhancement of AMPAR EPSCs, peripheral-nerve injury leads to an increase in responses mediated by GluN2B-containing NMDARs[33,104] (FIG. 3). Furthermore, in models of persistent inflammation, the expression of GluN2B subunits in the ACC is upregulated, thereby increasing the GluN2B component in NMDAR-mediated responses. Administration of GluN2B-receptor-selective antagonists, either systemically or directly to the ACC, inhibits pain hypersensitivity associated with peripheral inflammation. Interestingly, recent studies of the insular cortex and PFC have shown that upregulation of GluN2B-containing NMDARs after nerve injury also occurs in these structures[105,106]. This may therefore constitute a generalized cortical response to peripheral injury that facilitates the induction of NMDAR-dependent LTP. Such metaplasticity could contribute to the sustained enhancement of synaptic transmission that is associated with the chronic pain state.

Metabotropic glutamate receptors. The loss of LTD in the ACC that occurs in response to digit amputation can be rapidly rescued by addition of an mGluR agonist. This form of metaplasticity involves activation of PKC (FIG. 2).

Enhanced glutamate release. In addition to postsynaptic changes, the release of glutamate is also enhanced in the ACC in animal models of chronic pain[100,101,107] (FIG. 3). Paired-pulse facilitation is reduced in the ACC, indicating presynaptic enhancement of excitatory synaptic transmission, 1–2 weeks after peripheral nerve ligation (a model of neuropathic pain) or 3–5 days after CFA injection into the

hindpaw in adult mice (a model of inflammatory pain). Furthermore, an increase in the frequency of AMPAR-mediated miniature EPSCs occurs in ACC neurons after peripheral nerve injury[99] or inflammation in mice[100].

Altered intrinsic properties. In addition to changes in synaptic transmission, long-term changes in firing patterns and in intrinsic electrical properties have been noted in ACC neurons in chronic pain states[108,109]. There are at least three major types of pyramidal cell in the ACC, and these can be classified according to their action potential firing pattern: regular-spiking cells, intrinsic-bursting cells and intermediate cells. The population distribution and the single action potential properties of these three groups are not affected 1–2 weeks after nerve injury in mice[108]. However, intermediate cells from animals with neuropathic pain showed higher initial firing frequency[108]. Furthermore, it has been shown that the temporal precision of action potential firing in the ACC is reduced 1–3 days after injection of CFA or 1–2 weeks after nerve injury, as reflected in increased jitter[109]. These findings suggest that chronic pain does not cause dramatic changes in the frequency of ACC neuronal firing, but that the temporal precision of information coding in the ACC is reduced for long periods of time.

Intracellular signalling. Genetic and pharmacological manipulation studies have been used to investigate the molecular mechanisms underlying peripheral injury-induced changes in synaptic plasticity in the ACC. One pathway that might be involved is cAMP signalling. Genetic deletion of AC1 in mice abolishes or reduces allodynia-like responses in models of neuropathic pain, chronic inflammation and chronic muscle pain[110,111]. Furthermore, peripheral injury triggers phosphorylation of GluA1 subunits at the PKA phosphorylation site Ser845, as well as an increase in the expression of GluA1 subunits and enhanced AMPAR-mediated EPSCs, as measured 1–2 weeks later in the ACC[99]. Overexpression of CREB in forebrain neurons, including in the ACC, leads to enhanced LTP and behavioural nociceptive responses in murine models of neuropathic and inflammatory pain[112].

An atypical PKC isoform (either PKMζ or PKCι/λ) is crucial for maintaining enhanced synaptic responses in the ACC in rodent models of neuropathic pain[54]. Peripheral-nerve injury causes activation of PKMζ in the ACC, and locally applied ZIP erases synaptic potentiation. In addition, microinjection of ZIP into the ACC blocks mechanical allodynia in mice[54]. These observations suggest that chronic pain activates signalling cascades that are similar to those activated by experimental stimuli used to induce NMDAR-dependent LTP at ACC synapses.

In addition to inducing postsynaptic changes in AMPAR function, several studies suggest that the cAMP signalling pathway is important in the long-lasting enhancement of glutamate release in the ACC. In mice lacking AC1, the changes in paired-pulse facilitation and miniature EPSCs that are usually caused by nerve injury are absent[99,100]. Using a chemicogenetic approach in mice, it was found that selective activation of the cAMP pathway in the ACC *in vitro* enhanced glutamatergic synaptic transmission, and *in vivo* activation of the cAMP pathway in the ACC enhanced the behavioural responses to inflammatory pain[113].

Intracellular signalling pathways underlying the upregulation of GluN2B subunits in chronic pain have also been investigated in rodent models. Interestingly, AC1 is also required for the upregulation of the GluN2B subunit in the ACC that is caused by peripheral inflammation[33]. Caveolin 1, a protein that is associated with membrane lipid rafts, has also been shown to contribute to the upregulation of GluN2B subunits in the ACC after nerve injury[104].

To summarize, it seems highly likely that AC1-triggered cAMP signalling is involved in the synaptic changes in the ACC that are associated with chronic pain conditions.

Temporal considerations

The transient hyperactivation of the ACC in acute pain and its persistent hyperactivation in chronic pain in humans[6–8] are mirrored in findings from animal studies. At the synaptic level, it has been shown that exposure to transient noxious heat does not cause alterations in either presynaptic LTP or postsynaptic LTP in the murine ACC, whereas neuropathic injury affects both forms of LTP[54,59]. Although one might therefore conclude that enhanced transmission in the ACC is associated with chronic but not acute pain, it is likely that the relationship between LTP in the ACC and pain processing is more complex than this simple dissociation suggests.

For example, although acute nociception is short-lasting, it can trigger persistent synaptic changes associated with the formation of fear memory, a form of learning that has been widely studied in rodents[25,26]. There is evidence that synaptic plasticity in the amygdala[25,26,114] and in the ACC[115,116] supports fear memory (BOX 1). Thus, acute pain may engage synaptic plastic mechanisms in the ACC to encode physiologically relevant information, even if it is not directly related to chronic pain. A second consideration is that some forms of chronic pain may involve the continued presence of a noxious stimulus: for example, pain that is associated with the growth of a tumour. The extent to which synaptic plasticity is engaged in such forms of pain is unknown.

Another complicating factor is that LTP in pain pathways may, as occurs in hippocampal pathways subserving spatial memory, be subject to repeated renewal through the process of reconsolidation. Indeed, a recent study of capsaicin-induced mechanical allodynia in mice has revealed that a reconsolidation-like process occurs in the dorsal horn of the spinal cord. Here, a second application of capsaicin to the skin, administered simultaneously with intrathecal injection of a blocker of NMDARs or of protein synthesis, resulted in the failure of the pain memory to be reconsolidated and the consequent abolition of allodynia[117,118]. It will be interesting to determine whether a similar reconsolidation process, which requires NMDAR-dependent and protein synthesis-dependent

Table 1 | **Drugs that modulate pain-related plasticity in the ACC in rodent models**

Drug type	Administration route	Effect on acute pain	Effect on chronic pain	Effect on plasticity in the ACC		Refs
				Pre-LTP	Post-LTP	
Gabapentin	Systemic	Reduced	Reduced or no effect	No data	No effect	55
TRPV1 inhibitors	Systemic	Reduced	No effect	No data	No effect	147
GluN2B antagonists	Local ACC; systemic	No effect	Reduced	No effect	Reduced	33
AC1 inhibitor (NB001)	Local ACC; systemic	No effect	Reduced	Reduced	Reduced	55,68
PKMζ inhibitor	Local ACC	No effect	Reduced	No effect	Reduced	54
HCN channel (I_h channel) inhibitor	Local ACC	No effect	Reduced	Reduced	No data	59

AC1, adenylyl cyclase 1; ACC, anterior cingulate cortex; HCN, hyperpolarization-activated cyclic nucleotide-gated; LTP, long-term potentiation; PKMζ, protein kinase Mζ; TRPV1, transient receptor potential cation channel subfamily V member 1.

plasticity, also occurs in the ACC. If so, this might explain the analgesic effects of some drugs that are known to block the induction of LTP, such as GluN2B or AC1 inhibitors[33,68].

Determining the roles of synaptic plasticity in the ACC in different forms of pain is not straightforward. One possibility is that the induction of LTP in the ACC is required for the physiological processing of painful stimuli, including fear memory, whereas dysregulated or excessive LTP in the ACC might contribute to the chronic pain state. Further work is required to define more precisely the relationship between LTP in the ACC and the different affective and temporal components of the response to pain.

Conclusions

Insights into cortical plasticity induced by peripheral injuries are prerequisites to understanding the neural basis of chronic pain, and how chronic pain affects mood and cognitive function in animal models and humans. The ACC, along with other less-studied regions, such as the insular cortex, has a crucial role in the affective component of chronic pain. In this Review, we have focused on the role of LTP and LTD in the ACC, and how these phenomena contribute to the development of the chronic pain state. Knowledge of the molecular mechanisms of presynaptic and postsynaptic LTP and LTD in the ACC may provide potential new targets for the treatment of pain (TABLE 1) and associated emotions, such as fear and anxiety.

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The authors declare no competing interests.