Structural plasticity and reorganisation in chronic pain

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Abstract | Chronic pain is not simply a temporal continuum of acute pain. Studies on functional plasticity in neural circuits of pain have provided mechanistic insights and linked various modulatory factors to a change in perception and behaviour. However, plasticity also occurs in the context of structural remodelling and reorganisation of synapses, cells and circuits, potentially contributing to the long-term nature of chronic pain. This Review discusses maladaptive structural plasticity in neural circuits of pain, spanning multiple anatomical and spatial scales in animal models and human patients, and addresses key questions on structure–function relationships.

Nociception
The sensing of stimuli that are potentially harmful to the body; the sensory component of pain.

Acute pain
A transient form of pain that is acutely associated with a nociceptive stimulus.

Chronic pain
A pain that persists for long periods of time and, in most cases, extends beyond the period of healing of the original insult or injury.

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Nociception and acute pain serve an important protective function in preventing tissue damage. However, pain can become chronic when maladaptive processes that are triggered by pathophysiological factors (such as neural injury, trauma, amputation, viral infection, inflammation, tumour growth, exposure to neurotoxins, autoimmune disease, vascular diseases, metabolic disorders or stress-related alterations) are exacerbated early on by a range of psychosocial variables. Indeed, chronic pain is a major cause of human suffering worldwide[1], especially because effective, specific and safe therapies have yet to be developed.

Despite several commonalities, chronic pain syndromes of different aetiologies can be mechanistically distinct and show different clinical manifestations. Chronic inflammatory and muscular pain disorders involve a constant ongoing stream of nociceptive inputs from the affected tissues to peripheral and central nociceptive pathways (BOX 1). Chronic neuropathic pain, on the other hand, is associated with an imbalance of activity in pathways that results from loss or interruption of physiological inputs due to lesions to peripheral or central neurons. Several clinical pain disorders involve inflammatory and neuropathic components.

A large body of converging evidence suggests that chronic pain is not simply a temporal extension of acute pain but involves distinct mechanisms. The transition of acute pain into a chronic disorder involves activity-dependent changes (that is, functional plasticity) at many different interconnected levels, ranging from the molecular to the network level, at several anatomical avenues in the nociceptive pathway[2,3]. This interconnectivity can explain why even small molecular changes, such as a single point mutation, can result in large changes at the behavioural or clinical levels that are caused by amplification along multiple scales of plasticity. Mechanisms involving functional plasticity have been studied extensively and have revealed a range of modulatory factors that change the sensory, emotional and cognitive components of pain (reviewed in REFS 2–7). However, recent data show that functional plasticity changes are accompanied by structural remodelling and reorganization of synapses, cells and circuits that can also occur at various anatomical and temporal scales[7–9], thereby further adding complexity and a large dynamic range, and potentially accounting for the development of pain that extends over longer periods of time. Structural remodelling of connections has not been studied as widely as functional plasticity, and it remains unclear whether it represents a cause or a consequence of chronic pain.

This Review aims to discuss the latest insights into structural reorganisation in nociceptive pathways related to the transition from acute to chronic pain, integrating analyses in human patients and animal models across microscopic and macroscopic scales. Importantly, we attempt to address how structural changes influence and/or cause functional changes and whether they can be targeted therapeutically.

Structural plasticity at synapses

Spinal presynaptic and postsynaptic changes

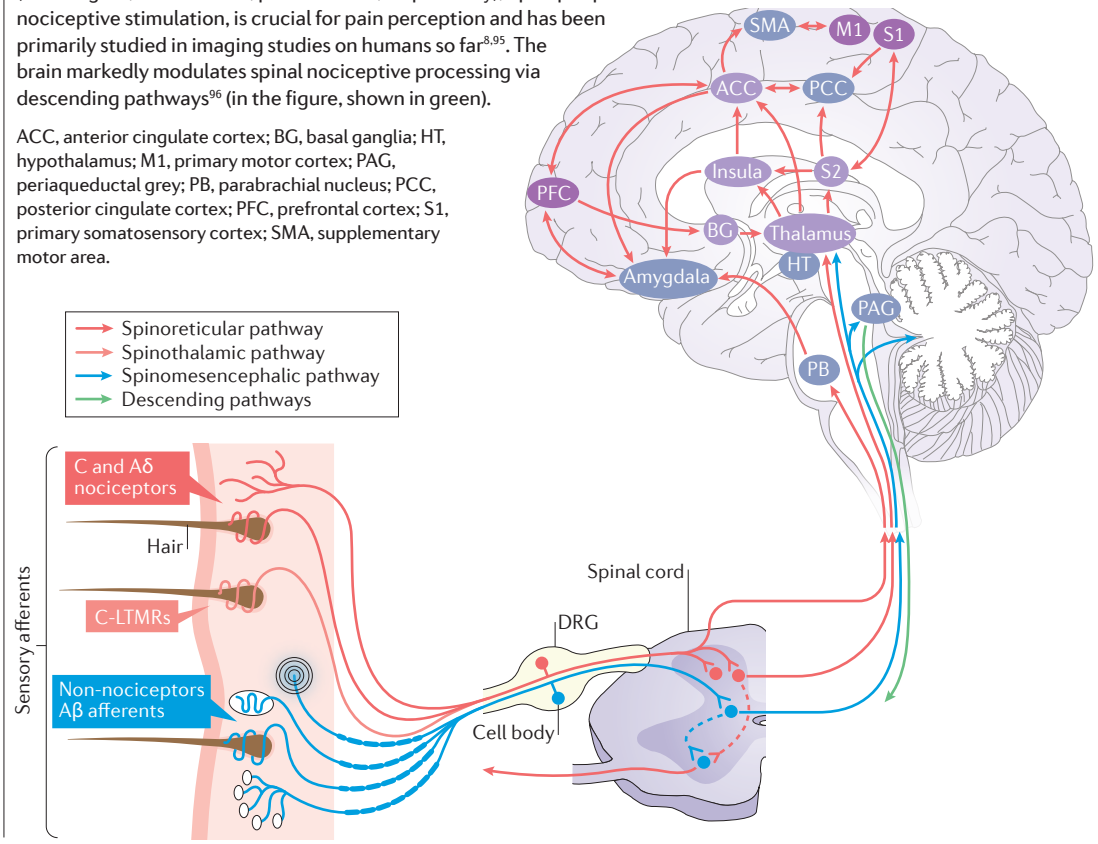
Homologous long-term potentiation (LTP) has been reported at spinal dorsal horn synapses between C-fibre (nociceptor) terminals and spinal neurons projecting to the brain (reviewed in REFS 3,10). It entails both presynaptic and postsynaptic mechanisms (reviewed in REFS 3,10,11) (FIG. 1a,b) that are thought to be caused by persistent activation of peripheral nociceptors following, for example, injury or inflammation. Synaptic LTP involves an increase in the probability of presynaptic

Box 1 | **Nociceptive pathways from periphery to brain**

Peripheral sensory neurons that are situated in the dorsal root ganglia (DRG) and trigeminal ganglia transduce and convey information about noxious and innocuous stimuli to the spinal dorsal horn and brain stem, in which nociceptive information is received, processed and modulated by descending control[3] (see the figure). Peripheral sensory afferents show diversity and specificity of function (such as C and Aδ nociceptive fibres, C-type low-threshold mechanoreceptors (C-LTMRs) and non-nociceptive amyloid-β (Aβ) afferents)[82] (see the figure). However, this specificity is lost in the spinal cord owing to convergence of inputs onto common subset of neurons[2,84]. Decoding how this information is segregated to enable delineating innocuous perception (such as touch and cooling) and pain has been one of the foremost challenges in the field[2]. High-threshold unmyelinated C-type nociceptor fibres and thinly myelinated Aδ-type nociceptor fibres transmit nociceptive signals mainly to neurons in spinal lamina I and outer lamina II, whereas low-threshold Aβ-type fibres transmit innocuous touch signals and synapse onto neurons in deeper spinal laminae, particularly lamina III[82]. A loss of this segregation is inherent to the clinically intractable symptoms of allodynia.

The brain, which harbours numerous cortical and subcortical structures that are activated via three major ascending pathways, spinoreticular, spinothalamic and spinomesencephalic pathways (in the figure, shown in red, pink and blue, respectively), upon peripheral nociceptive stimulation, is crucial for pain perception and has been primarily studied in imaging studies on humans so far[8,95]. The brain markedly modulates spinal nociceptive processing via descending pathways[96] (in the figure, shown in green).

ACC, anterior cingulate cortex; BG, basal ganglia; HT, hypothalamus; M1, primary motor cortex; PAG, periaqueductal grey; PB, parabrachial nucleus; PCC, posterior cingulate cortex; PFC, prefrontal cortex; S1, primary somatosensory cortex; SMA, supplementary motor area.



release at these synapses[11], and neurotransmitter release from C fibres is indeed increased in chronic pain models. Whether this is brought about by or related to structural modification is not known, and the time is now ripe to use recent advances in connectomics to elucidate the potential changes in presynaptic structure that accompany synaptic potentiation at spinal nociceptor terminals.

Postsynaptic mechanisms of spinal LTP are largely similar to those described in the hippocampus and involve NMDA receptor-dependent insertion and modulation of AMPA receptors[3,10]. Structurally, recent evidence suggests that the density of synaptic spines on dendritic segments increases in an activity-dependent manner in diverse models of chronic inflammatory and neuropathic pain, such as paw inflammation, diabetic neuropathy or chronic constriction injury (for examples, see REFS 12,13); altered spine structure and dynamics were also noted[14]. The stability of the actin cytoskeleton is governed mainly by molecular switches, RHO GTPases, which enable rapid association or disassembly of actin polymers in a temporally — and spatially — coordinated manner in response to extracellular cues. The RHO GTPase RAC1 favours actin polymerization and thereby stabilizes synaptic spines, whereas the molecule RHOA contracts actin via actin–myosin coupling and destabilizes spines[15]. Pharmacological studies in models of neuropathic pain suggest that RAC1 activity is required for increased spine formation in spinal neurons[13].

In a recent study, bidirectional genetic manipulation of the expression levels of RAC1 in excitatory neurons in the spinal dorsal horn revealed a close link between the density of synaptic spines on lamina II neurons and

Plasticity
The ability to change in an activity-dependent manner; it encompasses both structural and functional changes.

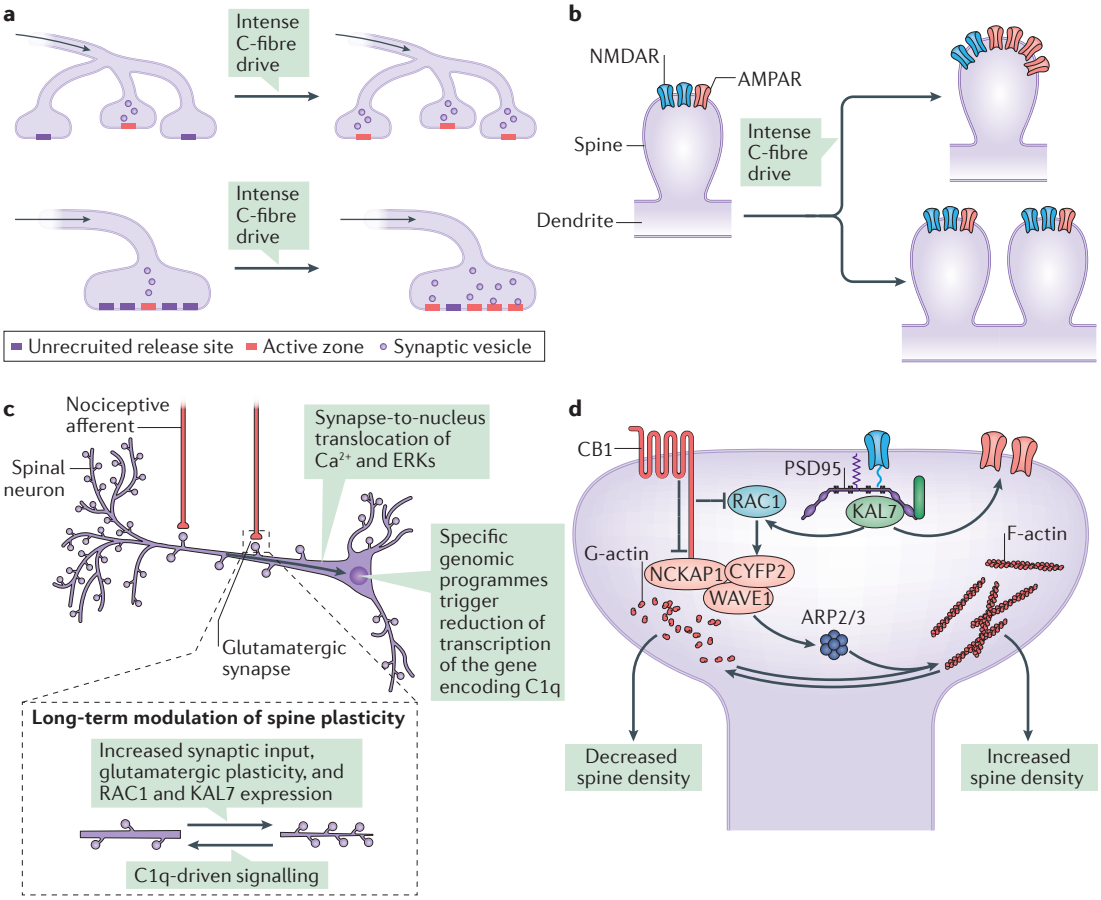


Figure 1 | **Nociceptive, activity-dependent presynaptic and postsynaptic plasticity at nociceptive synapses in spinal superficial laminae. a** | The schematics show a model of activity-induced increase in probability of presynaptic neurotransmitter release in small, single active-zone terminals of branched axons (top panel) or in a large terminal with multiple release sites (bottom panel). In this model, intense afferent C-fibre stimulation resulting from a painful stimulus that is detected by peripheral nociceptors results in an increase in active-zone recruitment. **b** | The schematic shows a model of changes in spine structure and postsynaptic glutamatergic receptor content (NMDA receptors (NMDAR) and AMPA receptors (AMPAR)) in which persistent nociceptive activity (intense C-fibre drive) can result in increased spine size or density of spines. **c** | At the synapse receiving input from peripheral nociceptors, postsynaptic messengers (such as extracellular signal-regulated kinases (ERKs)) travelling from the synapse to the nucleus of the spinal neuron trigger calcium signalling in the nucleus and activation of cyclic AMP-responsive element-binding protein (CREB)-dependent genomic programmes (such as reduced transcription of the gene encoding C1q), and thereby bring about long-term modulation of spine structure and density. **d** | Mechanisms of persistent nociceptive activity-induced remodelling of the actin cytoskeleton involving spine stabilization are shown. Nociceptive activity recruits intracellular kalirin 7 (KAL7)–RAC1 pathway via glutamatergic signalling, for example, via NMDARs, resulting in functional plasticity via AMPAR insertion and structural plasticity (either increased or decreased spine density, depending on which other components are active) via RAC1 signalling. This structural plasticity can be counteracted by therapeutically applied cannabinoids via physical interactions between cannabinoid receptor1 (CB1) and the WAVE1–RAC1 complex. ARP2/3, actin-related protein 2/3; CYFP2, cytoplasmic FMR1-interacting protein 2; NCKAP1, NCK-associated protein 1; PSD95, postsynaptic density protein 95.

the magnitude of inflammatory hypersensitivity[12]. The mechanism is likely to involve the protein kalirin 7, which binds to NMDA and AMPA receptors and plays a part in their synaptic localisation via postsynaptic density protein 95 (PSD95)–discs large homologue 1–zonula occludens 1 (PDZ)-domain interactions and is simultaneously capable of dynamically modulating the actin cytoskeleton via RAC1 activation (FIG. 1c,d). Disrupting interactions with kalirin 7 postsynaptically in spinal neurons attenuates inflammatory pain, abrogates spine remodelling and blocks the induction of nociceptive activity-induced LTP[12], thereby directly demonstrating that structural and functional plasticity at spinal synapses go hand in hand with induction of inflammatory hypersensitivity.

The maintenance of synaptic structural plasticity is likely to involve gene regulation. The second messengers cyclic AMP-dependent protein kinase A (PKA), extracellular signal-regulated kinases (ERKs) and calcium waves travelling from activated synapses to the neuronal nucleus have been hypothesized to account for the transition from acute to chronic pain[6,16] (FIG. 1c). Mechanistically, the specific genomic programme that is triggered by nuclear calcium in spinal neurons affects several genes encoding

cytoskeletal modulators, such as C1q, the initiator of the complement pathway, which acts as a synaptic pruning factor at spinal and hippocampal synapses[16]. In response to persistent presynaptic nociceptor activity, as in inflammatory pain states, postsynaptic nuclear calcium signalling transcriptionally suppresses the expression of C1q. This suppression of C1q expression has a permissive role in enabling activity-induced increases in spine density in the spinal dorsal horn, leading to hypersensitivity[16] (FIG. 1c). Thus, C1q-mediated synaptic pruning seems to counterbalance RAC1-mediated synaptogenesis in the spinal cord in a nociceptive activity-dependent manner. Interestingly, in contrast to inflammatory pain, interfering with the spinal nuclear calcium signalling pathway does not block neuropathic hypersensitivity, and a different gene regulatory programme seems to be operational in neuropathic pain. In neuropathic pain, apart from RAC1-mediated processes[13], synaptic remodelling may involve microglial mechanisms, given that prominent microglial activation is inherent to neuropathic states[6] and microglia mediate synaptic pruning during development[17]. Thus, the emerging picture is that activity-dependent structural remodelling of spinal dendritic spines has a causal role in the maintenance of chronic nociceptive hypersensitivity in both inflammatory and neuropathic pain states, although the molecular modes of how this is achieved may differ across different types of chronic pain.

In light of the potential therapeutic relevance of cannabinoids[18], it is interesting that cannabinoids suppress RAC1 activity in synaptic spines of adult spinal and cortical neurons by interacting with the WAVE1–RAC1 complex[19] (FIG. 1d). Cannabinoids were also shown to suppress nociceptive activity-driven remodelling of spinal dendritic spines and to concomitantly alleviate inflammatory pain[19]. This further supports the association between spinal dendritic spine remodelling and nociceptive hypersensitivity and suggests new avenues for therapeutic exploitation.

Spine changes in cortical neurons

LTP and other types of activity-dependent functional synaptic changes have also been reported in cortical areas that are associated with chronic pain, such as the insular cortex and the anterior cingulate cortex (ACC). Functional synaptic plasticity has been particularly well studied in the ACC, with reports on both presynaptic and postsynaptic contributions and increased AMPA receptor insertion (reviewed in REF. 20). However, whether these functional changes are accompanied by, or driven by, structural remodelling of spines has not been studied.

In the primary somatosensory cortex (S1), early phases of neuropathic pain are associated with an increased turnover of superficial spines, involving a seemingly random gain and loss, followed by a restoration of physiological turnover rates during later stages[21]. Furthermore, blockade of peripheral nerve activity during early stages after neuropathy prevents changes in synaptic turnover in S1, suggesting an ongoing dependence on ectopic activity in peripheral nerves[21]. Changes in spine stability were also recently reported in randomly tested dendritic segments in S1 after spinal cord injury (SCI)[22]. Spine remodelling also occurs in the medial prefrontal cortex (mPFC) of neuropathic mice[23]. These studies suggest that representation maps are altered in the cortex after loss of normal inputs or through gain of ectopic or aberrant inputs. However, elucidation of distinct types of neurons and spines affected, temporal analyses and causal contributions to neuropathic pain are still missing.

Cellular changes in circuits

Diverse types of chronic pain, such as chronic back pain[24], complex regional pain syndrome (CRPS)[25,26], fibromyalgia[27], rheumatoid arthritis[28] and post-amputation pain[29], have been broadly reported in patients to demonstrate macroscopic local morphological alterations in grey-matter density and volume in the brain[30], and more recently in the spinal cord[31], although the functional relevance of these changes is not clear. Studies are now beginning to emerge that recapitulate these abnormalities in animal models. Interestingly, in some cases, grey-matter changes are reversed after analgesic therapy[32]. This suggests that the cellular basis of these macroscopic level observations is given by highly dynamic structures, such as synaptic spines (discussed above), or glial cells that can divide rapidly, rather than neuronal somata. Here, we summarize insights from studies reporting changes in cell loss or gain in animal models of chronic pain.

Cell loss in the spinal cord

Reduced inhibitory drive in the spinal cord has been reported in models of neuropathic pain[33] and is broadly acknowledged as a plausible mechanistic basis for mechanical allodynia, but whether it results from an actual physical loss of inhibitory neurons is still disputed. Although some studies have reported caspase-dependent apoptosis in the spinal cord ipsilateral to the injured nerve[33,34], others have suggested that cell loss results from apoptotic microglia rather than apoptotic neurons[35]. Experimental ablation or silencing of spinal glycinergic neurons can induce allodynia[36]; however, stereological analyses in specific reporter lines have revealed no differences in glycinergic neurons in the spinal cord of neuropathic mice[37]. There are conflicting reports on potential changes in the number of GABAergic neurons, identified by immunoreactivity for GABA and GABA-synthesizing enzymes such as GAD65, in the spinal cord of neuropathic mice after nerve injury[33–35]. Similar models were compared across some studies, but it is possible that variations in the temporal and spatial domains that were analysed underlie these differences. An important caveat of all analyses hitherto is that none of the studies has addressed dynamic alterations in cell populations in a longitudinal manner (for example, through *in vivo* imaging) (TABLE 1) in conjunction with pain behaviour in each neuropathic animal. Indeed, the biological variability inherent to 'snapshot-like', single, spatial and temporal representations may account for the discrepancies that are reported across studies.

Given the importance of synaptic contacts, it is noteworthy that a detailed study was conducted on GAD65-expressing terminals and synapses, rather than

Chronic back pain
A pain that is associated with the back and that lasts longer than the expected period of healing. It can have neuropathic or inflammatory components, or both, but, in many cases, has no clear aetiology.

Complex regional pain syndrome
(CRPS). A chronic type of pain that typically affects a limb after trauma or injury and that can have inflammatory and neuropathic components.

Post-amputation pain
Acute and chronic pain that is caused by amputations. It can include postoperative pain, pain in the residual limb and pain in the amputated limb, referred to as phantom limb pain.

Allodynia
A pain or unpleasant sensations in response to a normally innocuous stimulus, such as a tactile stimulation (mechanical allodynia) or a mild change in temperature (mostly cold allodynia).

Pain behaviour
Behavioural changes (occurring both in the context of animal models of pain and in humans experiencing pain) that are indicative of pain. They can be of a spontaneous, ongoing nature or evoked by application of a noxious or innocuous stimulus.

Hyperalgesia
An exaggerated sensitivity and perception of pain in response to nociceptive stimuli.

GABAergic somata[38]. The authors reported a specific reduction in inhibitory terminals in the ipsilateral lamina II 3–4 weeks after injury in the chronic constriction injury model, which matched the functional reduction in GABA release that was reported previously[33]. At later time points, partial recovery of GABAergic terminals was observed. This stage-dependent loss was supported by observations in the spinal nerve lesion model in rats, in which distortion and loss of neurons, as well as a general sponginess in the spinal grey matter ipsilateral to the injury, were reported after 2 weeks and tapered off at later stages[39]. Thus, an initial transient loss of GABAergic inhibitory contacts corresponds to the induction of neuropathic pain, but this mechanism is unlikely to contribute to peak levels and chronic maintenance of neuropathic pain. Moreover, these morphological changes by themselves may not be sufficient for inducing neuropathic pain, because, in stereological studies[40], the loss of GABA-immunoreactive profiles was common to rats that behaviourally demonstrated either hyperalgesia or sensory loss after nerve transection. Moreover, a similar magnitude of GABAergic loss was observed across partial and total transections, indicating that small nerve injuries can have disproportionate consequences on the cytoarchitecture of spinal circuits. This has been suggested to account for the clinical observations on disproportionate pain that develops across different types and gradations of nerve injury[40].

Structural plasticity of inhibitory neurons may have therapeutic implications. Recent studies show that GABAergic precursor cells transplanted into the spinal cord rapidly differentiate into inhibitory neurons, structurally integrate into spinal circuits and develop inhibitory synapses onto spinal neurons. This was found to be effective in functionally bolstering inhibition and alleviating allodynia in neuropathic mice[41], as well as in alleviating itch, paving the way for potential therapies.

Neuronal gain via adult neurogenesis

Adult neurogenesis is one of the most important and exciting insights that has been uncovered in the field of neuroscience over the past decade. Very recent studies have shown a major link between hippocampal neurogenesis and pain. It has been reported that hippocampal neurogenesis of dentate granule cells is attenuated in mice with nerve injury[42]. Among the various stages of neurogenesis, proliferation of neuroblasts in the subventricular zone and survival of newborn neurons are negatively affected in neuropathic pain[43]. Although unequivocal causal links between these phenomena and chronic pain remain to be established, it is noteworthy that changes in hippocampal neurogenesis may correspond to affective changes that are inherent to the maintenance of chronic pain and its comorbidities, such as cognitive and affective decline. They seem to outlive allodynia but to coincide temporally with impairment of short-term plasticity, an inability to extinguish contextual fear, increased anxiety-like behaviour and a depression-like state that includes anhedonia and weight loss in animal models of neuropathic pain[44]. Interestingly, in line with clinical observations showing that stress exacerbates pain chronicity in humans, deficits in hippocampal neurogenesis in neuropathic rats were increased by simultaneously applied stress paradigms[43].

Table 1 | **Recent technological advances providing insights into structure–function relationships in pain-mediating pathways**

Model	Key advantages
Rodent	
Multiphoton imaging	Permits the study of fluorescently labelled structures or functional changes (calcium transients) in deep-seated, strongly scattering tissues in a living, intact organism in a minimally invasive manner with reduced photo-damage and lack of out-of-focus bleaching
In vivo multielectrode and tetrode recordings	Permits the study of potentials simultaneously in many brain areas (for example, via multielectrode arrays) or the delineation of single-cell activity (for example, via tetrodes) alone or in combination with optogenetic activation or silencing (for example, via optrodes) in awake, behaving animals
Optogenetic activation and silencing	Enables acute, short-term, light-induced activation or silencing of cells through the expression of excitatory (for example, channelrhodopsin) or inhibitory (for example, archeorhodopsin) opsins in a minimally invasive, reversible manner with a high temporal precision in awake, behaving animals
Pharmacogenetic activation and silencing	Enables minimally invasive, reversible and chronic, long-term silencing or activation in awake, behaving animals through the expression of excitatory or inhibitory designer receptors exclusively activating designer drugs (inhibitory DREADDs)
Human studies	
Neurofeedback	Uses brain activation patterns that are fed back to the person by brain–computer interfaces and helps to determine the relevance of a brain region for pain perception
Transcranial magnetic stimulation (TMS)	Uses non-invasive magnetic stimulation to induce inhibition or excitation and can thus interrupt or increase the activity in a certain brain region or in a circuit
Pharmacological manipulations	Uses specific receptor ligands (such as agonists or antagonists) combined with imaging techniques such as positron emission tomography (PET) to enable the study of transmitter systems
Operant and respondent learning and stress-induction paradigms	Can aid in delineating fear-, reward-, stress- or depression-related circuits
Virtual- and augmented-reality applications	Can aid in the induction of illusions or provide feedback from body representations and movements to delineate the role of body representation in pain

Structural changes in the hippocampus have also been reported in humans with chronic pain. A bilateral decrease in hippocampal volume was observed in patients with chronic back pain and CRPS but not in patients with osteoarthritis, and was accompanied by affective dysfunction and cognitive decline[45]. Moreover, patients with subacute pain, which became chronic, showed a decreased connectivity of hippocampal and prefrontal regions over time, which is consistent with the hypothesis that emotional learning and, specifically, extinction might be impaired in those individuals who move on to a chronic pain state[46].

However, the key question remains whether microscopic changes in neurogenesis or cell loss in animal models are mechanistically related to macroscopic level observations of brain volume reduction in human patients. A good start in this direction would be to integrate microscopic and macroscopic levels of analyses (for example, *in vivo* microscopy and structural MRI) (TABLE 1) in each animal into longitudinal studies in models of chronic neuropathic pain.

Structure–function changes in glia

Glia–neuron interactions are widely recognized to have a central role in nociceptive hypersensitivity[6]. Microglia, astrocytes and satellite glial cells (SGCs) show proliferation and remarkable structural changes, such as somatic hypertrophy, in diverse pain states. In the periphery, SGCs surround individual sensory neurons and enable neuron–neuron communication in the dorsal root ganglia (DRG) via glial gap-junction coupling, which is potentiated in models of visceral and neuropathic pain concurrently to SGC hypertrophy[47,48]. Functional studies indicate that blocking gap junctions in the DRG attenuates hypersensitivity, suggesting an important structural–functional link for SGCs in pathological pain[48].

In the spinal cord, functional contributions, rather than structural plasticity, of glia seem to be important. Recent studies have clarified that the structural hypertrophy and proliferation of microglia and astrocytes that are observed in chronic pain models are not functionally linked to nociceptive hypersensitivity, unless they are accompanied by neurochemical changes that modulate synaptic transmission and neuronal excitability in the spinal cord[6]. This is achieved through the release of diverse glia-derived mediators, which have been extensively reviewed recently (for examples, see REFS 6,17).

The involvement of glial activation and structural plasticity in human chronic pain was first detected in post-mortem analyses of patients with HIV, which indicated that only patients with painful neuropathy showed increased astrocyte proliferation and activation[49]. A recent technological breakthrough in integrated positron emission tomography (PET)–MRI in humans, in conjunction with a newly developed radioligand, now enables non-invasive live imaging of translocator protein (TSPO), which is believed to be a marker for activated microglia and reactive astrocytes[50]. In patients with chronic back pain with and without neuropathic involvement, higher TSPO-uptake values were detected in multiple brain regions, including the thalamus and the somatosensory cortex[50], paving the way for improved correlative and causal studies in humans.

Finally, a recent study has shown that the function of oligodendrocytes, a glial cell type that has not been widely studied in pain, is crucial for maintaining axonal integrity in the spinothalamic tract. Genetically induced ablation of oligodendrocytes in mice evokes classical symptoms of neuropathic pain long before demyelination begins[51]. This is important in light of the macroscopic white-matter tract defects and maladaptive regenerative plasticity that are observed in patients with chronic pain (for examples, see REF. 52), which may be indicative of oligodendrocyte dysfunction.

Network level changes

A network analysis[53] suggested that there are broad-ranging, as well as specific, changes that are related to various chronic pain syndromes, with a focus on prefrontal regions, the anterior insula, ACC, basal ganglia, thalamus, periaqueductal grey, post- and pre-central gyri and inferior parietal lobule. It has been reported that in younger patients with fibromyalgia, increases in grey matter are associated with higher pain thresholds, whereas, in older patients, grey-matter loss correlates with low pain thresholds, suggesting a shift from adaptive to maladaptive responses over time[54].

Resting-state networks

Studies on resting-state networks and their connectivity in patients with chronic pain have shown that chronic pain is associated with disrupted network properties, including failure to deactivate core regions of the default mode network (DMN) along with disrupted correlation and anti-correlation of selective regions of the DMN and attentional networks during a cognitive task[55]. A comparison of groups of patients with chronic back pain, CRPS and knee osteoarthritis[56], which show mixed components of inflammatory and neuropathic origin, showed similar changes in the DMN across all three groups. These included decreased connectivity of the mPFC with the posterior constituents of the DMN and increased connectivity with the insular cortex in proportion to the intensity of pain. Thus, the dynamics of the DMN seem to be reorganized in chronic pain, and this may reflect the maladaptive physiology of different types of chronic pain. In patients with fluctuating back pain, a shift towards higher frequencies in oscillatory activity during rest was found, as measured by functional MRI[4]. These enhanced fluctuations were confined to the mPFC and regions of the DMN. The shift in oscillatory activity was accompanied by changes in functional connectivity between the mPFC, insula, cingulate and secondary somatosensory cortex, with increases in clinical pain intensity coupled to these fluctuations. These data provide a link to the animal data that show the paramount significance of synchronous oscillatory activity for perception and provide a way to approach the question of the specific brain changes that are related to the perception of pain. MRI-based arterial spin labelling has been used to determine that increased evoked clinical pain correlates with changes in the connectivity of the DMN with the insula or ACC[57].

Painful neuropathy
A chronic pain that is causally associated with lesions of peripheral or central neurons.

Knee osteoarthritis
A pain that is caused by wearing away of the cartilage in knee joints.

Circuit changes in chronic pain

Although some studies have shown that functional and structural changes can be related to pain perception, many studies have reported divergent results. Functional imaging studies have yielded a good understanding of the brain regions that are involved in acute pain, and connectivity analyses have also shed light on the dynamic interactions of these regions in pain processing and in pain inhibition. However, their relation to structural changes needs to be further examined. When chronic pain was investigated, either experimental pain stimuli have been tested in patients with chronic pain or fluctuations in clinical pain have been examined. Using experimental pain stimulation several studies showed that chronic musculoskeletal pain is characterised by more intense and expanded brain activation patterns involving areas, such as somatosensory cortices, the insula or ACC, that tend to correlate with clinical pain duration[58] (reviewed in REF. 59) and by deficient activation of brain circuits that are involved in pain inhibition[60]. The insula has been identified as a core brain circuit involved in allodynia, but also implicated are the putamen, parietal and temporal regions of the cortex and thalamic circuits[61,62]. Decreases in the integrity of white-matter tracts have also been observed in patients with persistent chronic back pain[52].

Structural plasticity in injury states

Although maladaptive plasticity is established as a factor in pain chronicity, the specific nature of circuit alterations in diverse segments of the nociceptive pathway (BOX 1) is not well understood. Below, we discuss maladaptive structural changes at different anatomical stations.

Activity-dependent 'switches'

A 'phenotype switching' hypothesis has been postulated in which axotomized tactile-sensitive amyloid-β (Aβ) fibre neurons in the DRG undergo a change in their neurochemical signature that enables them to change their electrical properties, induce ectopic discharges in the DRG and transmit nociception[63]. Evidence for this view is provided by the observation that calcitonin gene-related peptide (CGRP), which is typically expressed in peptidergic nociceptors, is expressed *de novo* in a population of Aβ fibre neurons upon proximal or distal spinal nerve ligation (for examples, see REF. 64; but also see REF. 65). Functional links to chronic pain are given by observations that CGRP upregulation in large-diameter neurons is particularly pronounced in rat lines that are genetically selected for high neuropathic pain-like symptoms, as compared with rats with low neuropathic pain-like symptoms, and that a CGRP receptor antagonist attenuates neuropathic allodynia in these rats[64]. The recent development and launch of clinically useful CGRP receptor antagonists now enables the testing of the therapeutic relevance of these findings.

Remodelling of sympathetic fibres

Several studies report that sympathetic postganglionic efferents sprout peripherally in the skin after nerve injury and in the DRG, in form of pericellular basket structures surrounding DRG neurons, after proximal whole-nerve ligation or transection[66,67]. Despite early excitement, subsequent studies questioned the functional relevance of sympathetic sprouting to neuropathic pain, because similar morphological changes were observed in injured rats that showed well-developed neuropathic pain behaviour and rats that did not[68]. Moreover, in models involving distal partial damage or distal compression of nerves, analyses revealed that sympathetic sprouting is temporally delayed in comparison to early onset mechanical and cold allodynia after distal nerve injury[69]. These observations suggest that it is unlikely that sympathetic sprouting in the skin and DRG is causally linked to neuropathic allodynia.

Denervation of sensory axons

When nerve fibres are damaged, undamaged afferents from neighbouring territories can sprout into denervated areas (collateral sprouting), or damaged nerves can regenerate (regenerative plasticity). The most vivid example of structural plasticity in peripheral nerves is given by the formation of neuromas, bulb-like specialized endings that follow complete nerve transection, confer tremendous hypersensitivity and can elicit spontaneous pain by their ability to generate ectopic activity[63].

Traumatic injuries involving partial injuries to nerves are clinically far more common than complete nerve transections. In models of partial or spared nerve injury, collateral growth of uninjured axons from neighbouring nerves into the denervated areas and an increase in their density and branching in their native uninjured zone, that is, in the area showing allodynia, have been reported. However, a causal relation of these changes to allodynia is controversial, with both positive and negative evidence being reported[70–72]. Moreover, although some researchers reported excessive sprouting of CGRP-expressing peptidergic nociceptors in the uninjured, allodynic region in neuropathic animals, other investigators found no changes (for examples, see REF. 70) or even reported a decrease in the density of innervation[71,72]. Intra-ganglionic sprouting of CGRP-expressing axons in form of rings around non-nociceptive large-diameter DRG neurons after sciatic nerve transection has also been discussed controversially[65,73]. Furthermore, owing to a temporal mismatch between reported changes in afferent sprouting (delayed) and neuropathic allodynia (early onset), as well as owing to the correlative nature of post-mortem studies on biopsies, it remains unclear whether delayed sprouting actually underlies the chronic component of pathological pain or whether it represents a compensatory response to overcome sensory abnormalities.

Maladaptive regenerative sprouting of damaged axons, which results in aberrations in peripheral connectivity, represents a very attractive hypothesis for late (chronic) phases of neuropathic pain[74]. However, the functional relation to pain is ambiguous. In rats, treadmill exercise after injury has been shown to slow the rate of collateral and regenerative sprouting and attenuate hyperalgesia in the uninjured territory[75]. By contrast, some clinical studies suggest that nerve regeneration is closely related to the disappearance of pain and recovery of normal sensation[76].

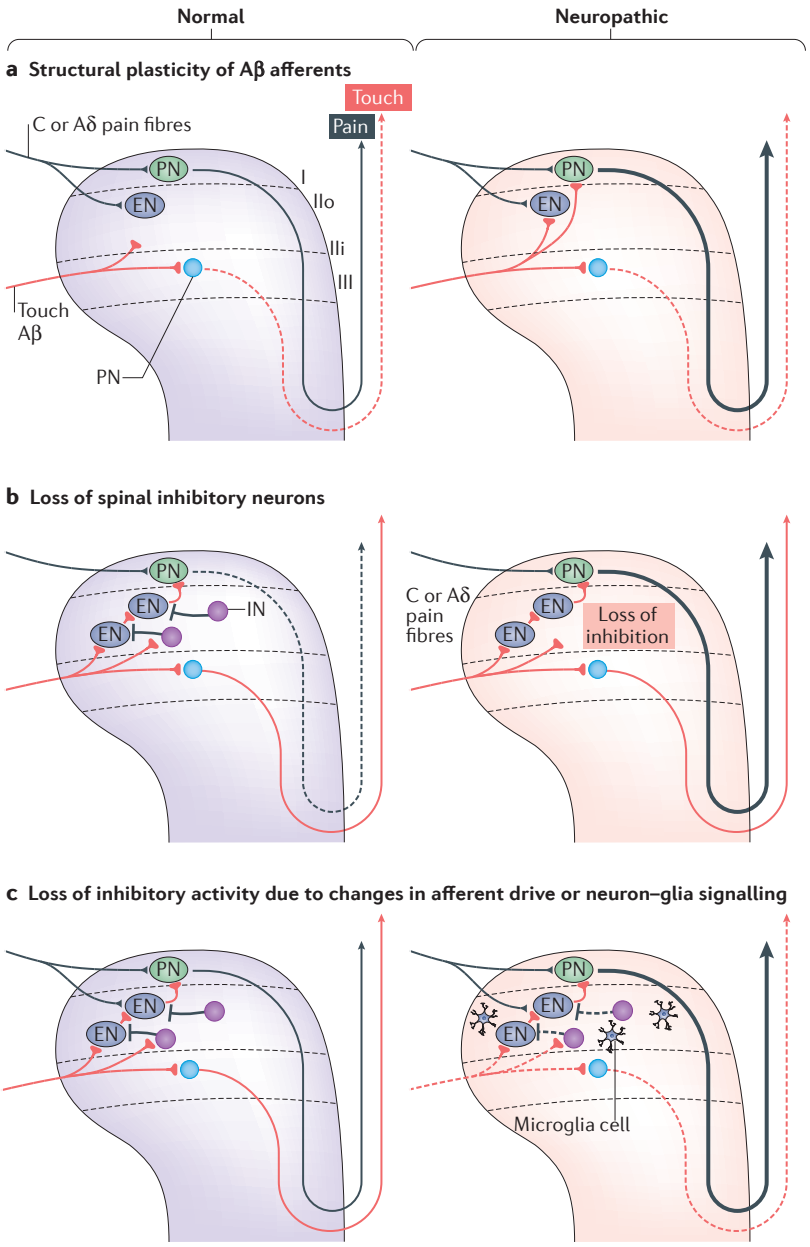


Figure 2 | **Models of changes in spinal circuitry segregating pain from innocuous touch in neuropathic mechanical allodynia. a** | In one model, touch-sensitive low-threshold mechanoreceptive fibres (Aβ fibres) that normally terminate in deeper spinal laminae (inner lamina II (IIi) and lamina III) sprout under neuropathic conditions into superficial laminae that typically receive noxious inputs (C fibres and/or Aδ nociceptive fibres) resulting in enhanced activity in spinal pain pathways (indicated by thicker black line in the right panel relative to the left panel). **b** | Spinal neurons receiving touch inputs are polysynaptically connected to spinal neurons receiving noxious inputs, and these polysynaptic connections are normally subject to strong inhibition. In neuropathic states, disinhibition through a physical loss of spinal inhibitory neurons can activate crosstalk between touch and pain circuits and result in increased activity in spinal pain pathways. **c** | Alternative models for disinhibition in neuropathic states involve defects in the structure or activity of low-threshold mechanoreceptive fibres, which would normally recruit spinal inhibitory interneurons, or proliferation and activation of spinal glia, which modulate activity of spinal excitatory and inhibitory neurons via secreted mediators. Both types of changes would lead to an imbalance in the spinal circuits that segregate touch and pain, where activity in spinal pain pathways is increased and activity in spinal touch pathways is reduced (indicated by the dashed red line). IIo, outer lamina II; EN, excitatory interneuron; IN, inhibitory interneuron; PN, projection neuron.

Thus, the concepts of both regenerative and collateral sprouting in neuropathic pain states require and deserve further evaluation and clarification, not least, owing to their potential therapeutic significance in peripheral targeting of pain, which may enable the circumventing of the central side effects of current medications. Learning from the limitations of previous analyses, future studies that are designed to unravel the course and functional importance of structural plasticity of peripheral nerves should ideally involve longitudinal non-invasive imaging studies, concomitant assessment of nerve structure and function, experiments testing causal contributions of nerve remodelling and approaches that delineate potential differences in the contribution of distinct types of afferents (TABLE 1).

Unlike in neuropathic pain, structural plasticity of peripheral sensory nerves has been more consistently reported, and structure–function links have been more clearly documented in cancer-associated pain. In models of bone metastatic pain, progressive tumour growth is temporally coordinated with evoked hypersensitivity, ongoing pain and sprouting of periosteal and skin afferents in the vicinity of tumour cells[77–79]; these changes are also mirrored in biopsy samples from patients with cancer, particularly in the context of visceral nerves in painful pancreatic carcinoma (for examples, see REFS 78,79). Do these changes contribute to cancer pain? Indeed, there is growing evidence that tumour-derived growth factors and cytokines, such as nerve growth factor (NGF), vascular endothelial growth factor and haematopoietic growth factors drive sensory nerve sprouting through receptor tyrosine kinase signalling and that blocking growth factor signalling inhibits cancer pain and structural plasticity of nerves in a temporally — and mechanistically — concerted manner[77–79].

Remodelling of spinal circuits

A loss of spinal segregation of touch (predominantly deep laminae) and pain (predominantly superficial laminae) has been hypothesized to be a key mechanistic element of the clinically intractable symptom of allodynia (FIG. 2). Conceptually, desegregation of touch and pain may arise at the level of spinal processing through at least two distinct, but not mutually exclusive, mechanisms: structural remodelling of circuits to enable physical links between nociceptive and non-nociceptive neurons (FIG. 2a) or disinhibition of existing physical links[80] (FIG. 2b,c). There is a large body of evidence supporting the latter, which can involve loss of inhibitory neurons (FIG. 2b), as noted above, and changes in activity of inhibitory pathways via various mechanisms, prominently including a loss of chloride-mediated inhibition that is mediated by downregulation of K–Cl co-transporter 2 (KCC2; also known as SLC12A5) in spinal neurons in response to brain-derived neurotrophic factor (BDNF) release from microglia[2,80,81] (FIG. 2c). Alternatively, a change in the nature of incoming afferent activity (for example, through the above-discussed alterations in peripheral neurons) can disturb the balance between spinal excitation and inhibition via deregulation of spinal interneurons (FIG. 2c). Indeed, very recent studies have

Bone metastatic pain
A class of cancer-associated pain that is caused by metastatic tumour growth in skeletal bones.

thrown light on the identity of spinal neurons and circuits that gate pain and/or innocuous touch (reviewed in REFS 2,82–84).

Although allodynia can be rapidly evoked by acutely neutralizing endogenous inhibitory control in the absence of structural remodelling, it does not exclude the possibility that structural reorganisation can take place in pathophysiological states and contribute functionally to the chronic nature of pain. Early observations from experiments on cholera toxin B-based tracing of large myelinated cutaneous (Aβ) afferents suggested that their central arborizations ectopically sprout into the nociceptive inner lamina II after peripheral nerve injury in the dorsoventral plane, thereby bringing tactile-sensitive afferents in physical proximity of nociceptive neurons (FIG. 2a). In later studies on bulk-loading approaches and single-fibre analysis, peripheral nerve injury-induced central reorganisation of low-threshold Aβ myelinated afferents was not prominently observed in neuropathic animals (for examples, see REFS 65,85). A recent study has suggested that, after axotomy in the mouse[65], high-threshold myelinated nociceptors with large-diameter axons and 'flame-shaped' arborizations recurve and send collaterals in a widespread manner throughout the nociceptive inner lamina II. This may account for the morphological observations that were previously thought to represent low-threshold tactile-sensitive (Aβ) fibre sprouting. However, because these arborizations were reported to be already present before nerve injury[65], a functional significance in neuropathic allodynia is improbable. Altogether, the evidence for disinhibition of existing links between non-nociceptive and nociceptive circuits outweighs the evidence for central sprouting of afferents as a mechanism for allodynia in models of peripheral nerve injury.

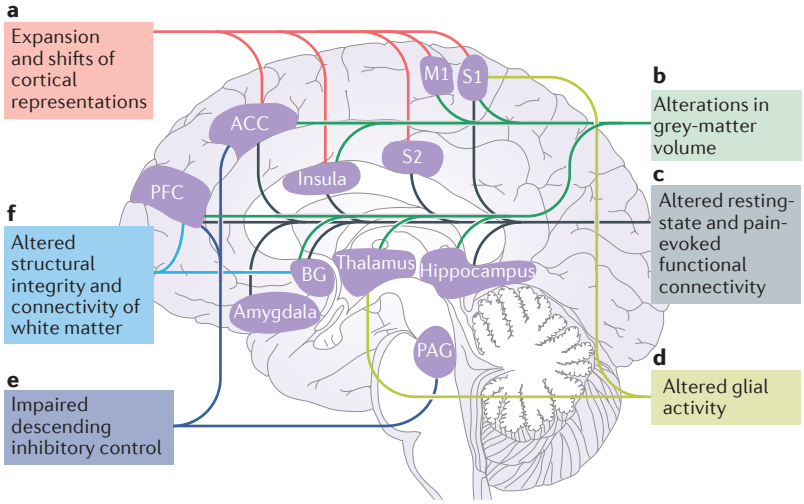


Figure 3 | **Structural and functional changes in the human brain in chronic pain conditions. a** | Brain areas undergoing functional reorganization. **b** | Regions of grey-matter alterations. **c** | Altered resting-state and pain-evoked functional connectivity. **d** | Brain glial activation. **e** | Changes in activity in descending inhibitory pathways. **f** | Changes in white-matter integrity and structural connectivity. ACC, anterior cingulate cortex; BG, basal ganglia; M1, primary motor cortex; PAG, periaqueductal grey; PFC, prefrontal cortex; S1, primary somatosensory cortex; S2, secondary somatosensory cortex.

In the emerging field of central neuropathic pain associated with SCI, sprouting of nociceptive afferents in the spinal cord has also been discussed. Whereas one study reported an increase in the density of non-peptidergic isolectin B4-binding nociceptive afferents in the contusion model of partial SCI in rats[86], another study showed an increase in peptidergic nociceptor labelling in lamina III–IV of the dorsal horn concurrent with the development of mechanical allodynia in mice, which was reduced upon treadmill training[87]. In future studies, it will be important to delineate the structural changes that are potentially caused by alterations in the expression of neurochemical agents that are used for visualising afferents and to dynamically image specific types of afferents.

Cortical reorganisation

Brain representation has been reported to shift from nociceptive to emotional circuits in chronic back pain[88]. On the basis of earlier animal studies on massive changes of cortical somatosensory maps as a consequence of amputation or deafferentation[89,90], a number of studies documented alterations (shifts) in the representation of sensory and motor maps in humans with phantom pain, pain related to CRPS and pain following SCI[9] (FIG. 3). These changes were found to correlate with the magnitude of perceived pain. Although this observation is supported by numerous studies[9], there is a current debate between the concepts of maladaptive cortical plasticity and persistent representation of the limb[91]. For example, it has been proposed that increased inputs into the cortical representation zone of the amputated limb, rather than reorganisation, is the cause of phantom pain in amputees when dorsal root ganglia or spinal inputs were blocked[92].

Some technical and experimental caveats, as well as the experimental context, must be considered in evaluating this conflicting literature. However, the most important point is that these scenarios are not necessarily mutually exclusive. A study based on a computational model of phantom limb pain[93] suggests that phantom pain, maladaptive reorganisation during tactile stimulation and persistent representation during phantom movements are related and driven by the same underlying mechanism, for example, an abnormally increased spontaneous activity of deafferented nociceptive channels. Furthermore, perceptual phenomena, such as telescoping or referred sensations in amputees, indicate that the representation of the former limb still exists and can be reactivated under appropriate stimulation conditions, for example, through mirror training[94]. This underlines a close association between the reduction in phantom pain and the normalization of the cortical representation. Therefore, key tasks for the future will be to document these changes longitudinally and elucidate causality related to pain (TABLE 1), which was not addressed in earlier studies.

Outlook and therapeutic implications

Overall, there is mounting evidence for structural plasticity and reorganisation in chronic pain (FIG. 3). We believe that it will be worthwhile to put focused efforts into achieving a profound understanding of causal relationships and the underlying mechanisms given the

tremendous boost that this knowledge can yield towards therapeutic development. We envision that these will not only span classical, molecular target-based interventions but may also encompass manipulations based on conditioning paradigms, neurofeedback and behavioural therapy, motor activity-dependent plasticity, peripheral and/or spinal neurostimulation and deep-brain stimulation.

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Competing interests statement
The authors declare no competing interests.