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Ionotropic Glutamate Receptors in Spinal Nociceptive Processing

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Abstract Glutamate is the predominant excitatory transmitter used by primary afferent synapses and intrinsic neurons in the spinal cord dorsal horn. Accordingly, ionotropic glutamate receptors mediate basal spinal transmission of sensory, including nociceptive, information that is relayed to supraspinal centers. However, it has become gradually more evident that these receptors are also crucially involved in short- and long-term plasticity of spinal nociceptive transmission, and that such plasticity have an important role in the pain hypersensitivity that may result from tissue or nerve injury. This review will cover recent findings on pre- and postsynaptic regulation of synaptic function by ionotropic glutamate receptors in the dorsal horn and how such mechanisms contribute to acute and chronic pain.

Keywords Hyperalgesia · Nociception · AMPA receptors · NMDA receptors · Kainate receptors · Long-term potentiation · Presynaptic regulation · Synaptic plasticity

Introduction

Nociception is the sensory modality, generally experienced as pain, that allows an organism to detect harmful or potentially harmful stimuli in peripheral tissue [1–3]. The perception of a stimulus, i.e., whether it is painful or not, is not an absolute property of the stimulus itself. For example, a normally innocuous stimulus may in some situations trigger activity in nociceptive circuits and be perceived as painful. Similarly, a normally nociceptive stimulus may sometimes be perceived as more painful than normal. These phenomena, referred to as allodynia and hyperalgesia, respectively, are attributed to a large part to central sensitization, the plasticity of neuronal excitability in sensory circuits of the spinal cord and supraspinal sites. This pain hypersensitivity serves an important physiological function by promoting behavior that protects injured tissue from further possibly damaging events, but may sometimes persist beyond the initial injury, in which case it is considered pathological. Furthermore, pathological pain may also develop as a consequence of neuropathy, i.e., injury in peripheral nerves or the central nervous system.

Glutamate was first reported to excite neurons of the dorsal horn (DH) of the spinal cord 50 years ago, although at the time the action was thought to be non-specific [4, 5]. However, it is now well established that primary afferent nerve fibers that convey sensory, including nociceptive, information from peripheral organs to the DH use glutamate as the primary transmitter [6–10]. Furthermore, most, if not all, excitatory interneurons within the DH are glutamatergic [8], as are corticospinal connections that terminate in the DH [7, 11, 12]. Thus, glutamate receptors in this region of the spinal cord play a crucial role in the integration of nociceptive signals and in their relay to supraspinal centers. These receptors not only mediate baseline responses to painful stimuli, but are also intimately involved in short- and long-term plasticity of the spinal nociceptive system that contribute to pain hypersensitivity. This review will consider the localization and function of ionotropic glutamate receptors (iGluRs) in the nociceptive circuitry of the mammalian DH and how their regulation may be involved in physiological and pathological pain states, with a focus on inflammatory and peripheral neuropathic pain.

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Spinal Nociceptive Circuitry

Most primary afferent fibers that carry nociceptive signals from the periphery to the spinal cord DH (and, in the case of craniofacial input, its equivalent structures in the trigeminal nuclei of the brainstem) are unmyelinated, slowly conducting C fibers or thinly myelinated, faster-conducting Aδ fibers, although a proportion of nociceptors are thickly myelinated Aα/β fibers conducting at high velocities [3, 13, 14]. It is also important to note that many C fibers and Aδ fibers are activated by low-threshold stimuli; thus, cells that receive C or Aδ fiber input are not necessarily nociceptive. Myelinated nociceptors respond to high-threshold mechanical or mechanical and thermal stimuli (and sometimes also to chemical stimuli) and give rise to a sharp pain sensation; they terminate throughout the DH, although Aδ nociceptors predominantly terminate in lamina I. Some Aδ fibers contain neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP). Many nociceptive C fibers are polymodal and respond to a wide range of stimuli, whereas others show a more selective stimulus sensitivity. Two parallel, albeit partially overlapping, nociceptive C fiber pathways have been distinguished mainly on the basis of neurochemical criteria (3, 14, 15, but see 16). The peptidergic population of C fibers expresses neuropeptides including SP and CGRP. The other population of nociceptive primary afferent fibers lacks or only sparsely expresses neuropeptides, but expresses the purinergic $P2X_3$ receptor and binding sites for the plant lectin isolectin B4 (IB4). In mice, the heat- and capsaicin-activated TRPV1 channel is expressed in few nonpeptidergic fibers [17–21]. Indeed, ablation of a large population of nonpeptidergic C fibers in mice selectively reduces mechanical nociception but leaves thermal nociception intact, and conversely, removal of spinal terminations of $TRPV1^+$ fibers results in reduced thermal nociception whereas mechanical nociception is unaffected [22]. However, the situation is more complex in the rat, in which many nonpeptidergic C fibers also express TRPV1 [16, 23–27] and respond to noxious thermal stimulation [28]. As a population, peptidergic fibers exhibit a broad innervation of both visceral and somatic tissue, whereas a large subset of nonpeptidergic fibers selectively innervates skin [3, 14, 19]. Peptidergic and nonpeptidergic fibers also show distinct termination patterns in the DH of the spinal cord. Nonpeptidergic fibers almost exclusively terminates in the dorsal part of inner lamina II (IIi), where they form the central terminals of type I glomeruli [3, 14, 29, 30]. Central terminations of peptidergic C fibers are mainly found in lamina I and outer lamina II (IIo). Nevertheless, despite the demonstrated neurochemical and anatomical differences between peptidergic and nonpeptidergic C fibers, it remains unclear to what extent they also have functionally distinct roles in nociception.

The spinal neuronal circuitry targeted by nociceptive primary afferent fibers is still relatively poorly understood, partly owing to difficulties in achieving coherent classification of neurons in the superficial DH [3, 14, 31]. Indeed, morphologically homogeneous populations may exhibit considerable heterogeneity in afferent input, electrophysiological properties, and transmitter phenotype and vice versa. Nevertheless, recent years have seen substantial progress in the efforts to elucidate the circuitry of the superficial DH (Fig. 1). Peptidergic afferents terminating in the superficial DH preferentially innervate lamina I neurons that express the neurokinin 1 receptor (NK1R) upon which SP acts, although they also contact $NK1R^-$ neurons [14, 32, 33]. Most projection neurons in lamina I are $NK1R^+$, and these are prominent targets of peptidergic C fibers. However, not only projection neurons but also interneurons in the superficial DH receive input from peptidergic fibers. A population of $NK1R^+$ projection neurons in laminae III–IV have dendrites extending dorsally that form synapses with peptidergic C fibers, but receive little direct input from nonpeptidergic C fibers or myelinated afferents [33]. It is unclear whether GABAergic cells receive input from peptidergic C fibers. Nonpeptidergic C fibers appear to innervate at least some lamina II interneurons that are classified as stalked or vertical cells [32]. Most such cells are excitatory and may form connections with both $NK1R^+$ and $NK1R^-$ lamina I neurons (although some are GABAergic [3, 34–36]); thus, nonpeptidergic C fibers may via this pathway converge with peptidergic C fibers (and possibly Aδ fibers) on lamina I output neurons (but see [37]). Furthermore, nonpeptidergic C fibers are also presynaptic to GABAergic (but not glycinergic) cells [38], some of which may be islet cells, and to a population of putative cholinergic/GABAergic cells in laminae IIi and III that may be involved in presynaptic inhibition of nonpeptidergic C fiber terminals [32, 39]. Notably, however, nonpeptidergic C fibers do not appear to contact the lamina IIi interneurons that express the γ isoform of protein kinase C (PKC), as was previously proposed [37, 40].

Structure and Function of iGluRs

There are three main classes of ionotropic glutamate receptors: the *N*-methyl-D-aspartate receptor (NMDAR), the α-amino-3-hydroxy-5-methyl-4-izoxazolepropionic acid receptor (AMPAR), and the kainate receptor (KAR). Of these, the AMPAR is the receptor primarily responsible for fast excitatory transmission at central glutamatergic synapses, whereas NMDARs and KARs generally have more modulatory roles. A new, more consistent nomenclature for iGluRs that was recently put forward by the International Union of

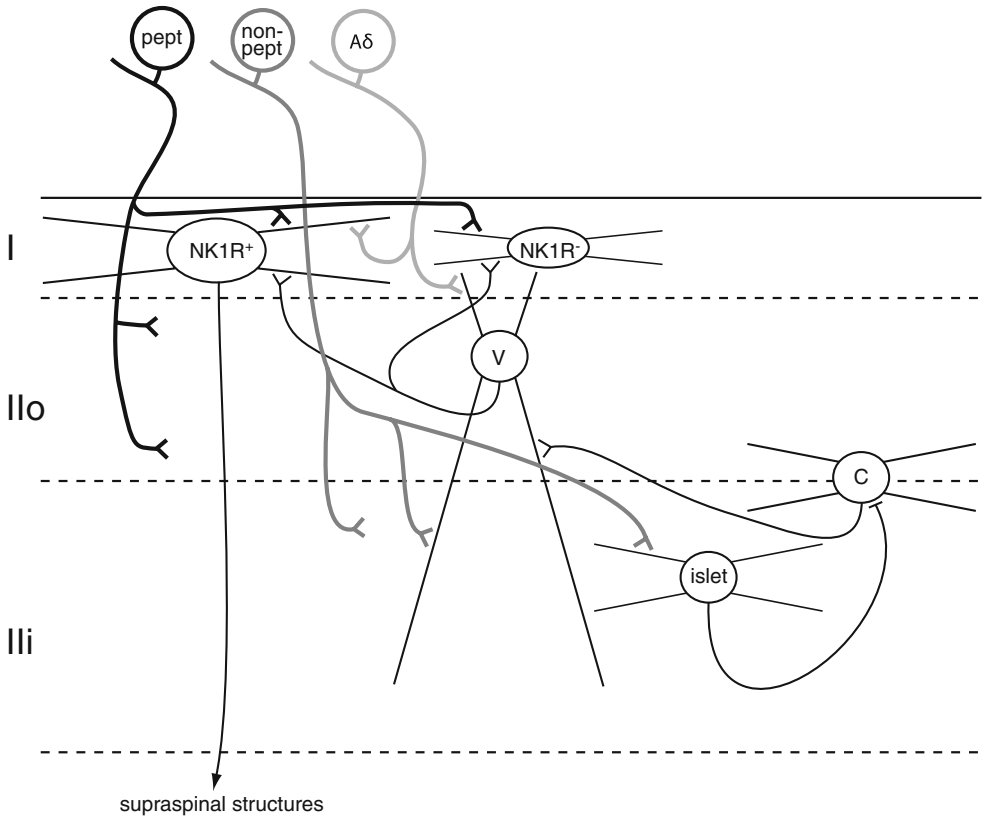

Fig. 1 Some components of the nociceptive circuitry the superficial DH. Peptidergic and nonpeptidergic C fibers and Aδ fibers have different laminar termination patterns and to some extent establish synapses with distinct neuronal populations in the DH. Peptidergic C fibers synapse with $NK1R^+$ and $NK1R^-$ projecting and nonprojecting lamina I neurons, as well as with dendrites in lamina II. Nonpeptidergic C fibers form connections with vertical neurons and islet neurons and with other neurons or dendrites in the dorsal half of inner lamina II. Such fibers may form disynaptic excitatory connections onto lamina I neurons via vertical cells. Aδ fibers target for instance lamina I $NK1R^+$ neurons and lamina II vertical neurons. *I* lamina I, *IIo* outer lamina II, *IIi* inner lamina II, *pept* peptidergic C fibers, *nonpept* nonpeptidergic C fibers, *V* vertical cells, *C* central cells; —| and —< indicate inhibitory and excitatory synapses, respectively

Basic and Clinical Pharmacology (IUPHAR) [41] will be used here.

NMDARs

The NMDAR is a tetrameric ion channel consisting of two GluN1 (also known as NR1 or GluRξ1) subunits in complex with two GluN2 or GluN3 subunits [42–44]. There are four different GluN2 subunits, termed GluN2A-D (previously NR2A-D or GluRε1-4), that each are encoded by a separate gene, and two different GluN3 subunits, termed GluN3A (previously termed NR3A, NMDAR-L or χ-1) and GluN3B (NR3B), similarly encoded by separate genes. In addition, alternative splicing of the GluN1 subunit yields eight possible variants of this subunit [45]. It has been suggested that some NMDARs contain two different GluN2 subunits, but the properties of such heterotrimeric receptors are unclear. GluN3 subunits may assemble together with GluN1 into heterodimeric receptors or with GluN1 and GluN2 subunits to form GluN1N2N3 receptors. The GluN1 subunit contains a binding site for glycine or D-serine, one of which is a required endogenous co-agonist of the NMDAR. The glutamate binding is situated in the GluN2 subunits, and at least one such subunit is therefore required for glutamate-activated function.

NMDARs possess several functional characteristics that are unique among ionotropic glutamate receptors and which are critical for the role of NMDARs in mediating synaptic and neuronal plasticity. Unlike most native AMPARs and KARs, NMDARs are generally highly permeable to Ca^{2+}, and exhibit much slower activation and deactivation kinetics. Furthermore, NMDAR activation is voltage-dependent because of a block of the channel pore by a Mg^{2+} ion at resting membrane potentials. NMDARs are also the only glutamate receptors activated by L-aspartate, which may be an endogenous neurotransmitter at some synapses [46, 47]. Notably, however, there appears to be no vesicular pool of L-aspartate in primary afferent terminals, arguing against a role for aspartate-mediated NMDAR activation in primary afferent transmission [48].

The properties of NMDARs are, to a large extent, determined by their subunit composition [44, 49]. GluN1N2A

receptors exhibit relatively fast deactivation (τ≈50 ms), whereas other GluN2 subunit-containing receptors deactivate much more slowly (GluN2A<GluN2B≈GluN2C≈GluN2D). GluN2A and GluN2B-containing receptors have high-conductance states that are highly sensitive to Mg^{2+}, whereas the conductance states of GluN2C and GluN2D-containing receptors are lower and less sensitive to Mg^{2+}. GluN3 subunits confer low Ca^{2+} permeability and single-channel conductance to the receptor. GluN1N3 receptors that lack a GluN2 subunit are not blocked by Mg^{2+} and are activated by glycine alone [44, 50, 51]. GluN1 splice variants also affect certain properties of the receptor, such as sensitivity to potentiation by polyamines, inhibition by Zn^{2+} or protons, as well as trafficking and surface expression [42, 44, 52].

AMPARs

Four genes encode the AMPAR subunits, termed GluA1-4 (also known as GluR1–4 or GluR-A–D) [49, 53]. Each subunit can be alternatively spliced in an extracellular region into either a flip or flop form, of which the latter is more prevalent in the adult. Flip/flop variability influence channel kinetics, pharmacology, and subunit stoichiometry. In addition, GluA2 and GluA4 are expressed with either long or short C-terminal tail; nearly all GluA2 subunits in the adult brain are short-tailed, whereas GluA4-long is the most common variant of this subunit. All GluA1 have a long C-terminal tail, whereas GluA3 is short-tailed. The tail variants determine important characteristics with respect to receptor trafficking: receptors containing short-tailed subunits such as GluA2-short and GluA3 are constitutively recycled to and from the postsynaptic membrane, whereas GluA1 and GluA4-long are recruited to synapses in an activity-dependent manner [54, 55]. Importantly, synaptic recruitment of GluA1 requires the activation of both Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and protein kinase A (PKA), whereas trafficking of GluA4-containing receptors to the postsynaptic membrane is dependent on PKA but independent of CaMKII.

An important posttranscriptional event is the RNA editing of Q607 to an Arg that nearly all GluA2 subunits undergo in the adult. AMPARs containing GluA2 edited at this site are impermeable to Ca^{2+}. Although most AMPARs contain GluA2, some do not, and such receptors are Ca^{2+}-permeable and exhibit inward rectification because of a voltage-dependent block by endogenous polyamines. Thereby, GluA2-lacking receptors may play a significant role in synaptic plasticity [56].

Compared to NMDARs, AMPARs exhibit fast-opening kinetics, and undergo rapid deactivation and desensitization [49]. However, the affinity of AMPARs for glutamate (~500 μM) is two magnitudes lower than that of NMDARs, whereas the affinity for L-aspartate is negligible [57].

KARs

The KAR family of subunits has five members, now termed GluK1–5 [41]; these are grouped into two subfamilies, the low-affinity (with respect to kainate binding) subunits GluK1-3 (also known as GluR5-7) and the high-affinity subunits GluK4-5 (KA1-2) [49, 58]. GluK1-3 form functional homo- and heteromeric receptors, whereas GluK4–5 are only functional when assembled together with any of the GluK1-3 subunits.

Alternative splicing yields a number of variant transcripts of GluK1-3. GluK1 and GluK2 are also subject to RNA editing. KARs containing GluK1/2 subunits edited in a Gln codon (similar to the Q607 site in GluA2) are impermeable to Ca^{2+} and have a very low single-channel conductance, whereas receptors with unedited subunits are slightly permeable to Ca^{2+}, have a ~25-fold higher single-channel conductance and are inwardly rectifying due to a voltage-dependent polyamine block [49, 58]. The proportion of unedited subunits decreases perinatally, but remains at a significant level in adult animals.

KARs have relatively similar kinetics to AMPARs except that they recover from deactivation considerably more slowly. This makes synaptic KARs less able to follow high-frequency presynaptic stimulation compared to AMPARs [49, 58].

Synaptic Localization of iGluRs

Postsynaptic Receptors

NMDARs

Postsynaptic proteins are often poorly detected by conventional immunohistochemistry, as epitopes within the dense protein meshwork of the postsynaptic density (PSD) are to a large extent inaccessible to antibodies. Thus, for example, NMDARs labeled using standard immunohistochemical methods are typically found predominantly in cell bodies rather than at synaptic sites [59, 60]. One approach to circumvent this problem is to treat the tissue with pepsin or some other proteolytic agent to degrade surrounding proteins, thereby revealing epitopes hidden within the PSD. Because many pre- and extrasynaptic membrane proteins are particularly sensitive to the proteolytic treatment, it is sometimes possible to use this approach to specifically examine postsynaptic receptor populations. Pepsin treatment was first used to detect postsynaptic NMDARs in the CA3 region of the hippocampus [61], and was subsequently applied by Todd and colleagues to label such receptors in the spinal cord [62]. They found that postsynaptic GluN1 subunits are rather evenly distributed

throughout the DH laminae. By contrast, postsynaptic GluN2A predominantly localizes to laminae III–IV and is sparser elsewhere. The GluN2B subunit shows an almost complementary postsynaptic distribution to that of GluN2A, with the strongest immunolabelling in the superficial laminae. Nagy et al. further reported that only about half of GluA2-containing puncta, most of which likely represent glutamatergic synapses [63, 64], in laminae I–II are immunopositive for GluN1 splice variants containing the C2′ cassette [62], which are the predominant splice variants in the spinal cord [65]. However, a recent study using freeze-fracture electron microscopy and immunogold labeling with an antibody presumably recognizing all splice variants found that nearly all (96%) of unidentified synapses in these laminae contain GluN1 [66], and that only a small proportion of synapses that contain AMPA receptors lack GluN1. This may suggest a preferential localization of C2 cassette-containing GluN1 splice variants at a subset of synapses in the superficial DH. Indeed, in situ hybridization studies indicate expression of such splice variants in the superficial DH [67, 68], and they have been detected by immunohistochemistry in DH neurons and neuropil at the light microscopic level [59, 69] and in synapses of the superficial laminae at the ultrastructural level [69, 70].

GluN1 is localized postsynaptically to central terminals of type I glomeruli [69] that originate from nonpeptidergic C fibers [29, 30]. GluN1 is also detected postsynaptically to most terminals containing SP [70]. Although some SP-containing terminals in the DH are of intrinsic spinal origin, and a small population may be terminations of descending pathways from the brainstem, most such terminals likely arise from nociceptive C or Aδ fibers that also express CGRP [29]. Thus, GluN1 may be present postsynaptically at most, if not all, synapses formed by peptidergic and nonpeptidergic nociceptive primary afferent fibers. Little is known about the postsynaptic localization of other NMDAR subunits in primary afferent synapses. The preponderance of GluN2B-positive synapses in laminae I–II together with the relatively low levels of postsynaptic GluN2A in these laminae suggest that NMDARs postsynaptic to nociceptive primary afferent fibers preferentially contain GluN2B subunits relative to GluN2A subunits [62]. In apparent contradiction to this, in lamina II neurons of adult rats, NMDAR-mediated excitatory postsynaptic currents (EPSCs) evoked by dorsal root stimulation are insensitive to the selective GluN2B antagonist ifenprodil, indicating the presence of GluN2A rather than GluN2B at primary afferent synapses onto these neurons [71]. This discrepancy has been proposed to be attributed to low-level activation of NMDARs by the stimulation protocol used, as ifenprodil is effective only at high glutamate concentrations [62, 72], but could also be partly explained by the presence of distinct NMDAR populations localizing to primary afferent synapses versus other glutamatergic synapses onto dendrites in lamina II.

Spinothalamic tract cells express GluN1 [73, 74], as do lamina I neurons that express NK1R [75]. Indeed, by single-cell RT-PCR it was found that of 37 superficial DH neurons sampled, all expressed GluN1 and at least one GluN2 subunit [76]. Moreover, nearly all neurons expressed more than one GluN2 subunit, and some expressed all four. GluN2A, GluN2B, and GluN2D were found in most superficial DH neurons, while GluN2C was the least abundant subunit [76]. Recently, an electrophysiological study of postnatal DH found that lamina I neurons expressing NK1R have a higher proportion of relatively Mg^{2+}-insensitive GluN2C/D-containing receptors than $NK1R^{-}$ lamina I neurons [77]. Furthermore, patch-clamp analysis of lamina II neurons indicates the existence of two populations of extrasynaptic NMDAR, one containing GluN2B and one containing GluN2D, as well as a synaptic receptor population containing GluN2A [71]. These observations indicate that all superficial DH neurons form functional NMDARs, and imply a considerable variability in the subunit composition of such receptors both within and between individual DH neurons.

AMPARs

Postsynaptic AMPARs containing the GluA2 subunit are present at essentially all glutamatergic synapses in the superficial laminae of the DH [63, 64, 66]. Thus, "silent" synapses that lack postsynaptic AMPARs, and therefore do not exhibit glutamatergic EPSCs at resting membrane potentials, are nearly absent in this region (see also 78). The majority of excitatory synapses in laminae I–II possess postsynaptic GluA1-containing receptors, whereas the proportion of GluA1-positive synapses is lower in lamina III [64, 66]. Immunofluorescence of GluA3 in pepsin-treated tissue has revealed that ~60% of synapses in the superficial DH contain this subunit, although immunoreactive puncta in these laminae are generally more weakly labeled than in deeper laminae [63, 64]. The long-tailed splice variant of GluA4 is found in ~25% of excitatory synapses in lamina I, but is sparse in lamina II, being present at less than 10% of GluA2-containing synapses in this lamina [64]. Interestingly, GluA1 and GluA4-long do not colocalize at synaptic sites in lamina I [64].

GluA1 is found at higher levels in synapses formed by thin-caliber primary afferent fibers in laminae I–II compared to synapses established by low-threshold primary afferent fibers in deeper laminae [63, 79, 80]. Moreover, GluA1 is found at a third of synapses formed by nonpeptidergic C fibers or peptidergic C/Aδ fibers, compared to 13–17% and 2–4% of synapses formed by VGLUT2-containing termi-

nals in laminae I–II and laminae III–IV, respectively [63]. As most VGLUT2-positive terminals in the DH arise from intrinsic DH neurons, it was suggested that GluA1-containing AMPARs are preferentially associated with primary afferent synapses.

Recent evidence suggest that GluA1 and GluA4 are present in distinct neuronal populations in the DH. $NK1R^+$ projection neurons in laminae III/IV, which have dendrites that extend into the superficial laminae, contain GluA2, GluA3, and GluA4 at postsynaptic sites, but lack GluA1 [81]. Lamina I spinothalamic neurons that lack NK1Rs also possess synaptic GluA4-containing AMPARs [82]. Among lamina I projection neurons that express NK1Rs, large cells tend to exhibit postsynaptic GluA4-containing AMPARs, whereas neurons with smaller somata have GluA1 (A. J. Todd, personal communication). Given the general paucity of synaptic GluA4 in superficial laminae and its enrichment in many DH neurons with supraspinal projections, it has been suggested that this subunit is predominantly found in such neurons, whereas interneurons instead express GluA1-, GluA2-, and GluA3-containing AMPARs [81].

KARs

Although in situ hybridization studies have reported only a weak expression of KAR subunits in the adult DH [83–85], KAR subunits have been detected by preembedding immunoelectron microscopy in postsynaptic dendrites in the spinal and trigeminal superficial DH [86–88]. Some GluK1/2/3-containing dendrites are postsynaptic to SP-containing terminals, at least some of which are probably of primary afferent origin [86], but in most instances the presynaptic partners of KAR-possessing dendrites have not been well described. Most dendritic preembedding immunolabeling of KARs is found over cytoplasm as opposed to the plasma membrane or PSDs [86, 87], although this could be partly because of an inability of the antibody to access the antigen within the PSD, as is known to be the case for other iGluR-specific antibodies (see above). Indeed, the postembedding immunogold technique, which suffers less from this limitation, has been used to demonstrate GluK1/2/3 subunit immunoreactivity over PSDs in the DH [87]. Nevertheless, a relatively extensive cytosolic localization of KARs in dendrites suggests that these receptors are not available for activation by synaptically released glutamate during basal conditions (see below).

Presynaptic Receptors

NMDARs

Presynaptic NMDARs in the DH were first described by Basbaum and colleagues, who observed presynaptic immunolabeling of GluN1 in the superficial DH, often in presumed nociceptive nonpeptidergic C fiber terminals of type I glomeruli [89]. Since then, several other groups have detected immunoreactivity for NMDAR subunits at presynaptic terminals in the DH, including terminals arising from myelinated low-threshold fibers and GABAergic interneurons [70, 73, 90–92]. GABAergic vesicle-containing profiles (terminals or dendrites) possessing GluN1 are sometimes pre- or postsynaptic to postsynaptic dendrites or central terminals of type I or II glomeruli, suggesting that GABAergic transmission in glomeruli may be regulated by glutamate spillover from the central terminal [91]. GluN1 was rarely detected in peptidergic primary afferent terminals in one study [90], although this could be because of splice variant-specific expression. A number of groups have also found various NMDAR subunits in DRG neuronal somata [89, 90, 93–99]. GluN2D is selectively expressed in peptidergic and IB4-binding small-diameter DRG neurons [94, 97].

AMPARs

AMPAR subunits are expressed in central terminals of both type I and type II glomeruli; the GluA4 subunit may be preferentially found in nonpeptidergic C fiber terminals, whereas GluA2/3 subunits may be more common in terminals of myelinated primary afferent fibers [100]. In the DRG, GluA4 is more commonly expressed in nonpeptidergic than peptidergic small-diameter neurons [99]. An antibody specific for GluA2/4 label ~25% of GABAergic terminals (identified by the presence of GAD65) in the DH, whereas two thirds of GluA2/4 immunopositive terminals are GABAergic [91]. By contrast, antibodies specific for GluA2 or GluA2/3 rarely produced immunolabeling in laminae I–II terminals or in DRG neurons, suggesting that many presynaptic AMPARs in the superficial DH are Ca^{2+}-permeable [91, 99, 100].

KARs

Among kainate receptor (KAR) subunits, the low-affinity subunit GluK1 and the high-affinity subunits GluK4 and GluK5 are found in central terminals of glomeruli, including many originating from nonpeptidergic C fibers, whereas KARs are rarer in peptidergic nociceptive primary afferent fibers [86–88]. KARs on nonpeptidergic DRG neurons are Ca^{2+} permeable early in development, but RNA editing of GluK1 in the DRG is substantially increased postnatally and onwards, resulting in the expression of Ca^{2+} impermeable KARs in postnatal and presumably also in adult nonpeptidergic DRG neurons [101]. KAR subunits are present in ~20–35% of GABAer-

gic terminals in the DH. Most KAR-containing inhibitory terminals form axosomatic or axodendritic synapses in simple configurations, although some KAR-containing GABAergic terminals or dendrites are part of type I or type II glomeruli [91].

Basal Synaptic Transmission

Responses to Electrical Stimulation

In superficial DH neurons, monosynaptic EPSPs/EPSCs evoked by electrical stimulation of C and/or Aδ fibers are almost completely blocked by AMPAR/KAR antagonists such as CNQX and its derivatives [3]. Similarly, monosynaptic EPSPs/EPSCs between connected neurons in the superficial DH are abolished by CNQX [34, 102, 103]. Antagonists of NMDARs only marginally affect the amplitude or rise time of monosynaptic C or Aδ fiber responses in laminae I–II neurons, but a slow component of the response is substantially reduced [3, 104, 105]. In conditions that relieve NMDARs of their Mg^{2+} block, such as at positive membrane potentials or in the absence of Mg^{2+}, an AP5-sensitive NMDAR-mediated component of C/Aδ fiber-evoked EPSCs is revealed in voltage-clamped laminae I–II neurons [104–106]. However, the proportion of the C fiber-evoked EPSC in lamina I neurons that is NMDAR-mediated decreases with age in rats, whereas the AMPAR-mediated portion shows a corresponding increase [104]. In lamina II neurons of adult rats, EPSCs evoked by intraspinal stimulation are never mediated solely by NMDARs, indicating the absence of silent synapses onto such neurons [78].

Electrophysiological studies using AMPAR-selective and KAR-selective antagonists or mice deficient in KAR subunits show that C/Aδ fiber-evoked EPSCs are almost entirely mediated by AMPARs and NMDARs in both lamina I and lamina II neurons [104, 105, 107, 108]. Furthermore, cultured DH neurons from young rats do not respond to a GluK1-selective agonist [109]. Still, there have also been reports of a small KAR-mediated component of primary afferent transmission in the superficial DH [110], and it has been suggested that Ca^{2+} influx through postsynaptic KARs contributes to primary afferent-evoked Ca^{2+} transients in lamina II neurons (see below) [111]. Thus, whether KARs participate in postsynaptic responses in nociceptive pathways of the DH remains unclear.

Ca^{2+}-permeable AMPARs that lack edited GluA2 subunits have been detected in neurons in all laminae of the DH using the cobalt loading technique [112, 113]. Accordingly, AMPAR-mediated primary afferent-evoked EPSCs in many lamina I $NK1R^+$ and $NK1R^-$ neurons and laminae III/IV $NK1R^+$ neurons show some, but variable, degree of inward rectification and sensitivity to antagonists of Ca^{2+}-permeable AMPARs, clearly indicating that many primary afferent synapses onto a substantial fraction of such neurons exhibit Ca^{2+}-permeable AMPARs [105, 107]. However, it should be noted that most of these observations were made in tissue slices obtained from immature animals. Indeed, a recent in vivo patch-clamp study in 8-week-old rats failed to detect a Ca^{2+}-permeable AMPAR-mediated component of pinch-evoked EPSCs in non-sensitized lamina II neurons [114]. Still, the sensitivity of some pain models to antagonists selective for Ca^{2+} permeable AMPARs (see below) indicate that such receptors are expressed, at least under some circumstances, also in the adult DH. Nevertheless, when present, Ca^{2+}-permeable AMPARs are probably intermingled with AMPARs impermeable to Ca^{2+} at primary afferent synapses, as GluA2 is found at nearly all excitatory synapses in the DH [63, 64, 66].

Polysynaptic pathways in the DH are probably mediated by both AMPARs and NMDARs [3]. Disinhibition induced by spinal administration of $GABA_A$/glycine receptor antagonists unveils polysynaptic Aβ fiber input to lamina I $NK1R^+$ neurons that is eliminated by NMDAR antagonism [115]. Similarly, spinal disinhibition facilitates polysynaptic Aδ/β fiber input to lamina II neurons in an NMDAR-dependent manner [116].

Responses to Natural Stimulation

Electrophysiology

As expected, inhibition of spinal AMPARs/KARs abolishes or substantially reduces responses in nociceptive-specific and wide-dynamic-range (WDR) neurons in the superficial and deep DH to noxious and innocuous mechanical and thermal stimuli [117–122]. The situation with respect to the role of NMDARs is less clear. Initial observations suggested that NMDARs are largely dispensable for the excitation of DH neurons by noxious stimulation [123]. However, some investigators reported that NMDAR antagonists attenuated responses of DH neurons to noxious, but only rarely to innocuous, stimuli [118, 119], whereas others observed a pronounced effect of NMDAR blockade also on responses to low-threshold stimulation [121, 124]. NMDAR inhibition does not prevent action potential firing in DH neurons, but attenuates a slow component of EPSPs evoked by natural stimulation, thereby reducing the number of action potentials fired [121]. However, it has been suggested that the effect of NMDAR antagonists on responses of DH neurons to natural stimulation observed by some may be attributed to an antagonist-induced general decrease in background activity of the neuron such that the threshold for primary afferent stimulation-induced excitation of the neuron is increased, rather than to the antagonist

acting directly on NMDARs in the primary afferent synapse itself [124]. Spinal administration of an antagonist selective for GluK1-containing KARs reduces responses in spinothalamic tract neurons to both innocuous and noxious mechanical (and possibly thermal) cutaneous stimuli in monkeys [125].

Nociceptive Behavior

Inhibition of spinal AMPARs is generally observed to induce antinociception [110, 126–132] (but see [133, 134]). Furthermore, spinal administration of AMPAR agonists induces vocalization responses indicative of pain in rats [135]. This is consistent with the large body of evidence indicating that glutamate and AMPARs mediate fast excitatory transmission at essentially all, if not all, primary afferent synapses as well as other excitatory synapses in the spinal cord [6–8, 10, 63, 66, 117–122]. Mice lacking the GluA1, GluA2, or GluA3 subunit of AMPARs exhibit no evident alteration in acute thermal or mechanical nociception, indicating that basal spinal nociceptive transmission is not sensitive to the subunit composition of AMPARs [113]. However, both GluA1- and GluA2-deficient mice show increased spontaneous nociceptive behavior in the first phase of the formalin test and after intradermal hindpaw capsaicin injection; as such behavior is thought to rely mainly on hyperactivity of primary afferent fibers, these observations were suggested to be due to an involvement of presynaptic AMPARs in the regulation of transmitter release from nociceptive primary afferent terminals (113; see below). Low-level inhibition of spinal Ca^{2+}-permeable AMPARs has been reported to produce slight acute thermal antinociception [136].

Eliminating functional postsynaptic NMDARs in the DH by region-specific knockdown of the obligatory GluN1 subunit does not affect acute nociceptive behavior [137–140], corroborating pharmacological evidence [127, 141, 142]. Further, knockdown or pharmacological inhibition of spinal GluN2B does not alter basal nociception [143–146], and mice deficient in GluN2A or GluN2D have normal acute nociception [147, 148].

Spinal inhibition of KARs or GluK1-containing KARs reduces behavioral or single-motor-unit responses to thermal or mechanical noxious stimuli [110, 130, 149]. However, activation of spinal GluK1-containing KARs, likely localized to primary afferent terminals (see below) may also result in antinociception [150]. Mice deficient in GluK1 or GluK2 behave normally in tests of acute thermal and mechanical nociception, but $GluK1^{-/-}$ mice exhibit reduced spontaneous nocifensive behavior both in early and late phases of the formalin test and after intradermal hindpaw capsaicin injection [151].

Short-Term Plasticity

Wind-up

Low-frequency electrical stimulation of C fibers induces a progressively increased excitability of many dorsal horn neurons, in particular WDR neurons in the deep DH that receive convergent nociceptive and low-threshold input, as well as of spinal reflexes. This facilitation, termed wind-up, subsides within minutes of cessation of the presynaptic stimulus. Although wind-up is often accompanied by at least some aspects of central sensitization, including receptive field expansion of WDR neurons, central sensitization may occur also without wind-up [152–154]. Thus, the physiological significance of wind-up is unclear. Nevertheless, dissecting the molecular mechanisms of wind-up may provide important insights into spinal nociceptive processing.

Although an increase in SP and/or glutamate release from primary afferent fibers has been proposed to contribute to wind-up, more attention has been given to potential postsynaptic mechanisms. Most studies have found wind-up to be partly dependent on activation of spinal NMDARs and NK1Rs [152]. However, some investigators have observed that wind-up in intermediate DH or both intermediate and deep DH neurons is not reduced and even facilitated by NMDAR antagonists in normal rats [155–157], but is attenuated by NMDAR inhibition in animals with peripheral inflammation [156]. Evidence from the spinal trigeminal nucleus also suggest that NMDARs may have either depressing or facilitatory effects on wind-up, depending on where in the network the affected receptors are located [157]. Furthermore, NMDAR inhibition may impair wind-up in spinal neurons that relay C fiber input to the primary somatosensory cortex [158].

Wind-up is relatively insensitive to AMPAR blockade in normal rats, although the sensitivity increases after carrageenan-induced hindpaw inflammation [159]. Mice deficient in the AMPAR subunits GluA1 or GluA2 exhibit normal wind-up [113]. An antagonist specific for GluK1-containing KARs reduces wind-up [159]; as a postsynaptic contribution of KARs to primary afferent transmission is uncertain (see above), it is possible that this effect is attributed to regulation of glutamate release by presynaptic KARs (see below).

It was initially proposed that presynaptic stimulation-induced AMPAR and neurokinin receptor activation sufficiently depolarizes the postsynaptic membrane to relieve some postsynaptic NMDARs of their voltage-dependent Mg^{2+} block, generating a slow EPSP. During repetitive stimulation, temporal summation of NMDAR EPSPs then occurs as progressively more NMDARs are unblocked, leading to a depolarized, hyperexcitable state of the neuron.

However, cumulative depolarization is neither necessary nor sufficient for wind-up, arguing against such a role for NMDARs in wind-up [152]. On the other hand, activation of L-type Ca^{2+} channels generate plateau potentials that may be involved in the expression of wind-up in turtle and rat deep-DH neurons [160, 161]. It was recently proposed that L-type Ca^{2+} channels are activated downstream of NMDARs in DH neurons but that under some circumstances, for example if inhibitory input is suppressed, activation of such Ca^{2+} channels and subsequent plateau potentials may take place also in the absence of NMDAR activation; this could help explain why NMDAR antagonists are sometimes ineffective in preventing wind-up [162].

Regulation of Transmitter Release

In the recent decade, it has become increasingly clear that presynaptic iGluRs, activated in an autocrine or paracrine manner, may also modulate release of glutamate and other neuroactive substances from DH synapses. However, it should be noted that, as it is difficult to ascertain a presynaptic locus of receptor activation in vivo, most studies have focused on cultured neurons or slices from young animals where, for example, an adult pattern of primary afferent termination has not been attained. Nevertheless, the prominent expression of presynaptic iGluRs in the spinal cord of adult rats (see above) suggests that similar mechanisms exist also in the mature animal.

One of the first indications of a presynaptic role for iGluRs in nociceptive transmission was the observation that intrathecal NMDA injection in awake rats leads to internalization of NK1Rs in the DH; this was attributed to an enhanced release of SP via direct activation of NMDARs localized on peptidergic primary afferent terminals [163]. However, this interpretation remains somewhat controversial; for example, a recent study found no evidence of an effect by intrathecal NMDA administration on basal or noxious stimulus-evoked NK1R internalization in the DH in anesthetized rats [164]. In addition, expression of NMDARs appears to be sparse in peptidergic primary afferent terminals [90].

Presynaptic iGluRs have been implicated in regulating glutamatergic transmission at primary afferent synapses, generally by reducing glutamate release from the terminal [108, 109, 165, 166]. The mechanisms underlying presynaptic iGluR-mediated regulation of glutamate release from primary afferent terminals are still relatively unclear; however, some potential mechanisms are outlined below and depicted schematically in Fig. 2. Activation of NMDARs present on A and C fiber terminals generates a primary afferent depolarization (PAD) in the dorsal root [165]. This may interfere with propagation of action potentials into the primary afferent terminals either via shunting caused by the increased membrane conductance, or via inactivation of voltage-dependent Na^+ channels. Alternatively, action-potential-evoked release may be inhibited by inactivation of voltage-dependent Ca^{2+} channels. However, the frequency of miniature EPSCs that reflect action-potential-independent vesicular release from lamina II synapses were also found to be depressed by NMDA, suggesting (although whether these synapses were of primary afferent origin could not be determined) that a direct influence on release machinery may also be involved [165]. Activation of presynaptic AMPARs depresses release of glutamate from primary afferent synapses onto lamina II neurons, perhaps via a PAD-associated mechanism [166]. Activation of presynaptic KARs that include the GluK1 subunit shows a biphasic effect on primary afferent glutamate release; whereas a high concentration of agonist depresses transmitter release [108, 109], a low agonist concentration has been reported to instead facilitate release [108]. Conceivably therefore, homo- and heterosynaptic activation of presynaptic KARs may have opposite effects on glutamate release. The facilitatory component may be attributed to an enhancement of exocytosis by weak depolarization of the presynaptic membrane, whereas the depression induced by strong KAR activation may be caused by a stronger depolarization (i.e., PAD) [108] or to G-protein-mediated inactivation of Ca^{2+} channels [167].

Not only release of glutamate but also of inhibitory transmitters may be regulated by presynaptic iGluRs (Fig. 2). Activation of GluK1-containing presynaptic KARs increases action potential-dependent and -independent GABA and glycine release at inhibitory synapses between DH neurons [168, 169]. This effect may be dependent on Na^+ influx through the receptor and subsequent activation of voltage-gated Ca^{2+} channels [168]. A GluK1 antagonist decreases action potential-dependent and -independent inhibitory transmission to lamina II neurons, suggesting that tonic low-level activation of KARs by endogenous glutamate may facilitate GABA/glycine release [169]. By contrast, Kerchner et al. [168] found that evoked inhibitory transmission in the superficial DH was attenuated when preceded by primary afferent stimulation. They suggested that glutamate released from primary afferent terminals activate KARs on adjacent inhibitory terminals, causing release of GABA that via activation of presynaptic $GABA_B$ receptors reduces further transmitter release from the same terminal. Thus, presynaptic KARs appear to have a biphasic effect on transmitter release from inhibitory terminals, in congruence with their role at primary afferent synapses. Presynaptic AMPARs enhance action-potential-independent GABA/glycine release onto lamina II neurons as well as onto NK1R-expressing lamina I neurons in both postnatal and more mature rats; this was suggested to be partly attributed to Ca^{2+} influx through Ca^{2+}-permeable AMPARs

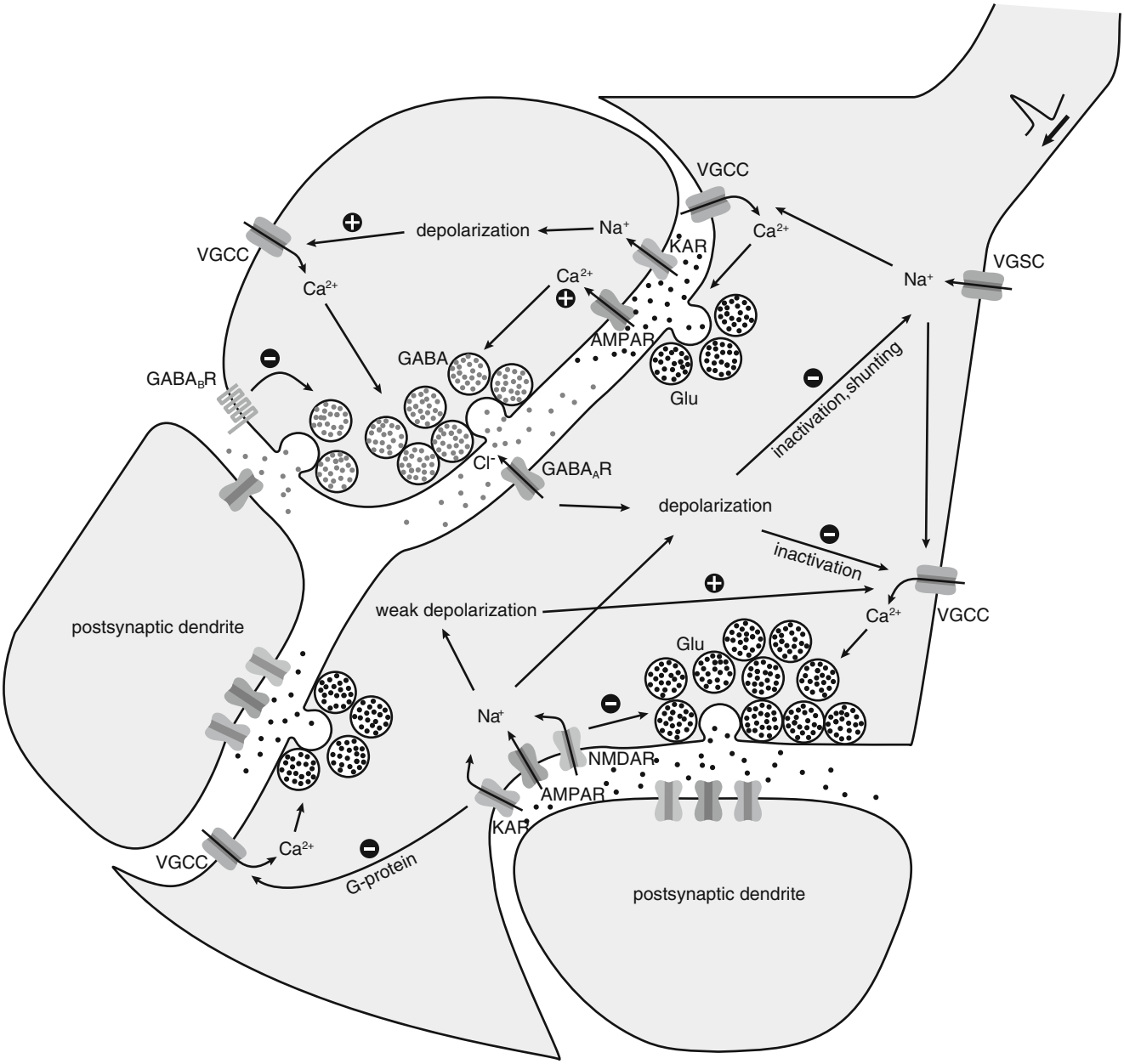

Fig. 2 Presynaptic regulation of glutamate and GABA release. Depicted is a type I glomerulus consisting of a central primary afferent terminal, postsynaptic dendrites and a GABAergic terminal forming reciprocal synapses with the central terminal as well as with a postsynaptic dendrite. See main text for details. *$GABA_AR$* $GABA_A$ receptor, *Glu* glutamate, *VGCC* voltage-gated Ca^{2+} channel, *VGSC* voltage-gated Na^+ channel. For other abbreviations, see text

[170]. However, similar to what has been observed for KARs, evoked inhibitory transmission was depressed rather than facilitated by AMPAR activation, although the underlying mechanism for this depression was not investigated [170]. In the same study, focal activation of AMPARs indicated that only a portion of inhibitory terminals are subject to AMPAR modulation, consistent with the observation of GluA2/4 immunoreactivity in about 30% of GABAergic terminals in laminae I–III [91].

Long-Term Plasticity

It has been known for quite some time that hyperalgesia induced by peripheral tissue injury is partly dependent on enhanced excitability of neurons in the spinal cord [171]. A number of different cellular mechanisms have been proposed to contribute to this central sensitization, including changes in neuronal circuitry and in membrane excitability (for a recent overview of such mechanisms, see [172]). One

class of mechanisms that has received much attention is the regulation of glutamatergic excitatory synaptic transmission in spinal sensory pathways. Indeed, ever since the first demonstration of a potentiation of primary afferent transmission akin to long-term potentiation (LTP) at the CA3–CA1 synapse in the hippocampus [173], such plasticity has been widely considered a candidate mechanism that may contribute to pain hypersensitivity [172, 174–177]. This notion has been reinforced by the observation that natural noxious stimulation induces a long-lasting potentiation of C fiber synapses in the intact animal [178] and by the recent series of studies by Treede and co-workers showing that cutaneous electrical stimulation of C fibers by protocols similar to those that induce LTP in animals produces enhanced pain perception in humans [179, 180].

LTP and Long-Term Depression (LTD)

Randić et al. first reported that strong high-frequency stimulation (HFS; several 100 Hz 1-s bursts) of the dorsal root induces either LTP or LTD of primary afferent synapses in the rat superficial DH in vitro [173]. Whether LTP or LTD was induced depended on the membrane potential of the postsynaptic cell as well as on the intensity of the conditioning stimulus; a more depolarized membrane and higher stimulus intensity favored LTP, whereas hyperpolarization and less intense stimuli favored LTD. Although most investigators have used HFS protocols to induce primary afferent LTP, nociceptive C fibers rarely fire at such high frequencies during natural stimulation; more common is an asynchronous firing at a frequency of ~1–10 Hz [176]. However, stimulation of C fibers at a low frequency (2 Hz) may also result in LTP of C fiber-evoked field potentials and of C fiber-evoked EPSCs in lamina I neurons that project to the periaqueductal gray (PAG) [178, 181, 182]. Furthermore, chemically induced excitation of cutaneous nociceptors by capsaicin or formalin produces LTP of C fiber-evoked field potentials in intact (i.e., non-spinalized) rats [178]. Thus, it is clear that physiologically relevant stimuli are capable of producing LTP in spinal sensory pathways. An additional form of long-term plasticity, spike timing-dependent plasticity (STDP), has also been recently described in primary afferent synapses on lamina II neurons in spinal cord slices from postnatal rats [183]. To induce STDP, a postsynaptic action potential is evoked by a depolarizing step within some milliseconds of presynaptic stimulation. The direction of STDP is dependent on the order of the pre- and postsynaptic stimuli; if the presynaptically induced EPSC precedes the postsynaptic action potential, LTP is normally induced, whereas LTD results if the stimulus order is reversed.

Changes in the rate or number of action potentials fired by a DH neuron in response to primary afferent stimulation have sometimes been used to study long-term plasticity of primary afferent synapses. However, it has recently been argued that this is not a good measure of primary afferent LTP or LTD, because the extent to which a DH neuron fires action potentials is determined by a number of different parameters, including membrane excitability and the strength of inhibitory and excitatory inputs to the neuron, in addition to the efficacy of activated primary afferent synapses impinging on the neuron [176]. Similar interpretative difficulties apply to optical imaging of spinal slices using bath-applied voltage-sensitive dyes, which reflects net tissue excitation including both EPSPs and action potentials [184]. However, primary afferent-evoked extracellular field potentials (which indicates summation of monosynaptic EPSPs as opposed to action potentials [185]) or monosynaptic EPSCs/EPSPs recorded from single DH cells may be used to assess the strength of primary afferent synapses. That said, increases in primary afferent-evoked firing of DH neurons may be a convenient indicator of sensitization of such neurons [186].

Many signaling molecules and their associated pathways that are known to be crucial for the expression of LTP at other central synapses have also been implicated in various forms of LTP of primary afferent synapses. Thus, it may be justified to construct a tentative model of some of the molecular mechanisms that underlie primary afferent LTP from the known molecular dependencies of such LTP while extrapolating to some extent from mechanisms of NMDAR-dependent LTP at other central synapses (Fig. 3). However, it should be emphasized that partly different mechanisms must underlie LTP at different populations of primary afferent synapse. First, different proteins involved in primary afferent LTP are differently expressed between synaptic populations; for example, NK1Rs are probably primarily associated with peptidergic rather than nonpeptidergic primary afferent synapses [33, 187] (see also above), and if LTP of nonpeptidergic C fiber synapses also requires NK1R activation, it can be reasonably assumed to be through some intermediate mechanism. Second, LTP at different primary afferent synapses differ in their dependencies on signaling pathways and type of conditioning stimulation. For example, in lamina I neurons retrogradely labeled from the parabrachial nucleus, HFS but not LFS of C fibers is capable of inducing LTP, whereas in lamina I neurons projecting to the PAG, LFS but not HFS induces LTP [178].

Induction Mechanisms

The induction of LTP of primary afferent synapses is generally observed to be critically dependent on the activation of NMDARs. This includes induction of LTP of C fiber-evoked field potentials by C fiber HFS or LFS in

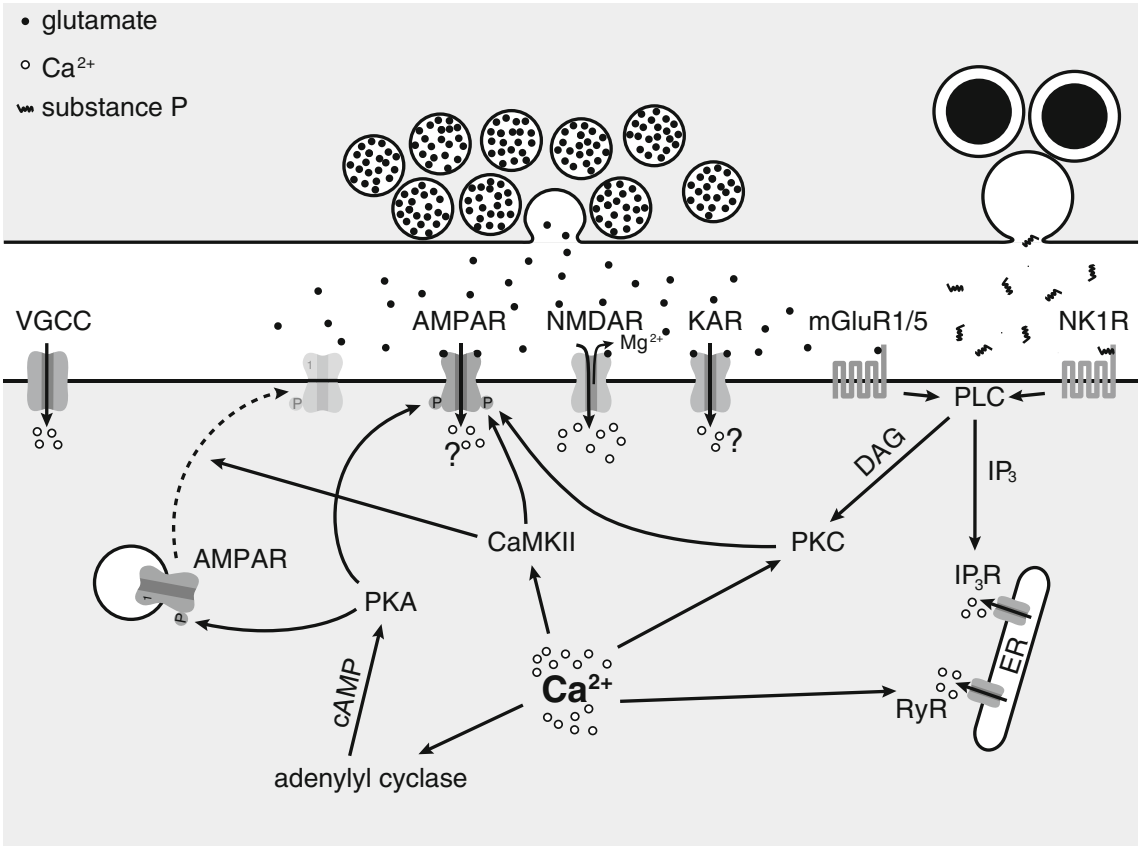

Fig. 3 Some mechanisms that may contribute to primary afferent LTP. Glutamate released from the primary afferent terminal act on AMPARs and group I mGluRs in the postsynaptic membrane. Postsynaptic NK1Rs are activated by substance P released from peptidergic primary afferent fibers. Depolarization of the postsynaptic membrane, for example by AMPAR activation, allow NMDARs to escape their Mg^{2+} block. Ca^{2+} entry through unblocked NMDARs, voltage-gated Ca^{2+} channels, possibly Ca^{2+}-permeable AMPARs and KARs contribute to a cytosolic Ca^{2+} transient, as do G_q-coupled receptors such as group I mGluRs (mGluR1/5) and NK1Rs through activation of phospholipase C and IP_3Rs. This may ultimately lead to potentiation of AMPAR- and NMDAR-mediated synaptic transmission by synaptic recruitment of such receptors and/or modulation of receptor properties by phosphorylation. Note that not all depicted mechanisms are shared by all primary afferent synapses that are able to undergo LTP. Within the depicted AMPARs, *1* denotes the GluA1 subunit. *cAMP* cyclic adenosine monophosphate, *DAG* diacylglycerol, *ER* endoplasmatic reticulum, *P* phosphate group, *RyR* ryanodine receptor, *VGCC* voltage-gated Ca^{2+} channel. For other abbreviations, see text

vitro or in vivo [178, 181, 188, 189], induction by HFS or LFS of LTP of C fiber-evoked EPSCs in lamina I projection neurons [178, 181, 188, 190], as well as primary afferent STDP induction in lamina II neurons [183]. However, at primary afferent synapses in the caudal trigeminal nucleus, a presynaptic form of LTP that is independent of iGluRs has been observed [191, 192]. A long-lasting LFS-induced, iGluR-independent potentiation of primary afferent excitability in the superficial DH has also been revealed using voltage-sensitive dyes loaded specifically into primary afferent fibers [184]. Spinal application of NMDA leads to LTP of C fiber-evoked field potentials in spinalized but not in intact rats, suggesting that tonic descending inhibition impedes opening of the voltage-dependent NMDARs [193]. Furthermore, HFS of Aδ fibers leads to an NMDAR-dependent LTP of C fiber-evoked field potentials in spinalized rats but to LTD (that is also dependent on NMDARs) in intact animals [194]. In spinal cord slices, LFS of Aδ fibers induces NMDAR-dependent Aδ fiber LTD in lamina II [195]. Thus, activation of NMDARs may be a crucial step in the induction of homo- or heterosynaptic plasticity of primary afferent synapses. Intriguingly however, intravenous administration of a low dose of ketamine prior to HFS of C fibers prevents primary hyperalgesia but not secondary hyperalgesia or allodynia in humans, suggesting that heterosynaptic potentiation in spinal sensory pathways is independent of NMDARs [180].

There are some indications that AMPARs take part in shaping the induction properties of LTP at primary afferent synapses. It was first noted that in some DH neurons in culture, activation of Ca^{2+}-permeable AMPARs led to short-term potentiation of synaptic transmission that sometimes lasted for the duration of the experiment (up to 15 min) [196]. Mice that are deficient in the GluA2 subunit

(and therefore only possess Ca^{2+}-permeable AMPARs) exhibit HFS-induced LTP of thin-caliber primary afferent synapses that is partly independent of NMDAR activation [197]. Thus, given the presence of Ca^{2+}-permeable AMPARs at primary afferent synapses in the superficial DH also in the normal animal (see above), it is possible that such receptors are involved in the induction of LTP at some primary afferent synapses.

Whether KARs are involved in LTP or LTD induction at primary afferent synapses has been studied in spinal cord slices from mice deficient in either the GluK1 or GluK2 subunit [111]. In mice lacking GluK1, partial LTP of monosynaptic C/Aδ fiber-evoked EPSPs in lamina II neurons can be induced by primary afferent HFS, but this LTP lasts only 20–25 min. By contrast, the same HFS protocol or an STDP protocol that both normally induce LTP of Aδ fiber synapses on lamina II neurons instead lead to LTD of these synapses in $GluK2^{-/-}$ mice. On the other hand, stimulation protocols that induce primary afferent LTD in wild-type mice also do so in both $GluK1^{-/-}$ and $GluK2^{-/-}$ mice. Furthermore, in wild-type and GluK1- but not GluK2-deficient mice, KAR activation generates a Ca^{2+} transient in lamina II neurons that is insensitive to blockade of voltage-gated Ca^{2+} channels, NMDARs and AMPARs (a possible contribution of internal Ca^{2+} stores was not investigated). Thus, it was proposed that KAR-induced Ca^{2+} transients in lamina II neurons may be mediated by postsynaptic GluK2-containing KARs, that in this manner contribute to the induction of LTP at thin-caliber primary afferent synapses.

Apart from iGluRs, a number of other ligand-gated receptors and voltage-gated ion channels on DH neurons have been implicated in the induction of different forms of primary afferent LTP and/or LTD. These include G_q protein-coupled receptors such as group I metabotropic glutamate receptors (mGluRs) [183, 198, 199] and NK1Rs [178, 181, 182, 190], receptor tyrosine kinases such as EphB receptors [189, 200] and the BDNF-activated trkB receptor [201], nitric oxide-activated soluble guanylate cyclase [178, 202], and T-type voltage-dependent Ca^{2+} channels [178, 190].

Signaling Pathways

NMDAR-dependent LTP at the CA3-CA1 synapse in the hippocampus is divided into an early phase lasting about 1–3 h that relies on posttranslational mechanisms such as iGluR phosphorylation and trafficking, and a late maintenance phase that is also dependent on translation of novel proteins [203]. Similarly, the development of LTP of C fiber-evoked field potentials induced by C fiber HFS is not prevented by prior administration of protein translation inhibitors, but the C fiber-evoked field potentials starts to decrease at ~2 h, and have returned to baseline within 5 h [204].

A massive body of evidence indicates that autophosphorylation of CaMKII at Thr286, and the Ca^{2+}-independent activity of the kinase that results from this phosphorylation, is a crucial step in the induction of early-phase NMDAR-dependent LTP at many if not most adult forebrain synapses where such LTP is inducible [205]. Not surprisingly then, a number of observations supporting a similar role for CaMKII in primary afferent LTP have been reported in recent years. Inhibiting CaMKII prevents induction of HFS- and LFS-induced LTP of C fiber-evoked field potentials and of LFS-induced LTP of monosynaptic C fiber-evoked EPSCs in lamina I spino-PAG neurons [178, 181, 206]. The requirement for CaMKII is time-dependent; a CaMKII inhibitor abolishes C fiber LTP when administered spinally 30 min but not 60 min after LTP induction [206]. This indicates a selective role for CaMKII in the early phase of primary afferent LTP. However, CaMKII has also been proposed to encode the efficacy of individual synapses in the maintenance of LTP and possibly also in basal states. This is based on the observation that CaMKII phosphorylated at Thr286 (pCaMKII) in the PSD is resistant to dephosphorylation by protein phosphatases, which suggests that the autophosphorylated state of CaMKII in the PSD may persist for years despite turnover of synaptic proteins, including CaMKII itself [207–209]. Why then, have CaMKII inhibition generally not been observed to affect the late phase of LTP in the spinal cord or elsewhere [206, 210]? The answer may be that the CaMKII inhibitors most commonly used compete with Ca^{2+}/calmodulin binding and therefore likely only affects Ca^{2+}-dependent CaMKII activity, whereas the Ca^{2+}-independent activity of pCaMKII is not inhibited. In support of this, a recent study found that a non-competitive CaMKII inhibitor that inhibits also Ca^{2+}-independent activity abolishes hippocampal LTP in the maintenance phase as well as reduces basal synaptic transmission [211].

Both PKC and protein kinase A (PKA) are necessary for the early phase of primary afferent LTP. Inhibition of either of these kinases prevents LTP of C fiber-evoked field potentials induced by HFS of C fibers in vivo [206]. The time course of the sensitivity of established primary afferent LTP to PKC or PKA inhibition is shorter than that to inhibition of CaMKII; already established LTP is reversed when kinase inhibitors are applied 15 min but not 30 min after LTP induction by HFS [206]. Similarly, C fiber LFS-induced LTP of C fiber-evoked field potentials or of monosynaptic C fiber-evoked EPSCs in lamina I spino-PAG neurons is inhibited by prior administration of a PKC antagonist [178, 181].

Phospholipase Cβ, which is activated by G_q protein-coupled receptors such as group I mGluRs [212] and NK1Rs [213], cleaves phosphatidylinositol 4,5-bisphosphate into diacylglycerol that activates PKC and inositol-1,4,5-trisphosphate (IP_3) that activates IP_3 receptors

(IP_3Rs). Indeed, phospholipase C (PLC) is required for HFS- and LFS-induced LTP of C fiber synapses in vivo and in vitro [178, 181, 190]. Inhibition of IP_3Rs, which release Ca^{2+} from intracellular stores, prevents HFS- and LFS-induced C fiber LTP [181, 190], and shifts the direction of LFS-induced plasticity to LTD in lamina I spino-PAG neurons [178]. Moreover, in lamina II neurons in young rats, an STDP protocol that normally induces primary afferent LTP instead results in LTD when PLC, IP_3Rs or group I mGluRs are blocked during the conditioning stimulation [183]. By contrast, activation of group I mGluRs alone induces LTD of Aδ fibers by a PLC-dependent mechanism [214]. Ryanodine receptors that mediate Ca^{2+}-induced Ca^{2+} release from internal stores are necessary for LFS-induced LTP of C fiber-evoked field potentials in the superficial DH [181], but not for primary afferent STDP in lamina II neurons [183]. LFS-induced Aδ fiber LTD in lamina II neurons is attenuated by a postsynaptic Ca^{2+} chelator, but is resistant to protein phosphatase 1 and 2A inhibition [195]. However, HFS-induced LTD of A fiber synapses in lamina II is dependent on both postsynaptic Ca^{2+} elevation and on protein phosphatases 1 and 2A [215]. These observations indicate that the precise spatiotemporal characteristics of the postsynaptic Ca^{2+} transient determine the polarity of primary afferent synaptic plasticity.

Expression Mechanisms

Early work suggested that primary afferent LTP and LTD involve strengthening of both AMPAR- and NMDAR-mediated transmission [173]. However, remarkably little is still known about the mechanisms that underlie the expression of these phenomena. For example, whether synaptic recruitment of iGluRs and/or modulation of the functional properties of iGluRs already present at the synapse contribute to primary afferent LTP and LTD has not been investigated. That said, more is known about the expression mechanisms underlying enhanced primary afferent transmission in different models of hyperalgesia (see below), and it is reasonable to hypothesize that similar processes are involved in electrically induced plasticity of primary afferent synapses.

Central Sensitization and Hyperalgesia

Induction Mechanisms

Spinal NMDARs, contrary to their minor role in acute nociception, have been shown in numerous studies to be essential for the development and maintenance of central sensitization and centrally mediated pain hypersensitivity in many models of persistent pain (e.g., [3, 137–141, 143, 144, 216]). Importantly, spatially restricted knockdown of GluN1 indicates that postsynaptically localized NMDARs in the DH are required for mechanical hyperalgesia and nocifensive behavior in inflammatory pain [137, 139, 140]. However, the dependence on NMDARs is not universal; for example, NMDAR antagonists do not affect the development of mechanical hyperalgesia in models of postoperative pain and burn injury [217, 218] (but see [216]). GluN2B-containing NMDARs may be particularly involved in some aspects of pain hypersensitivity. Spinal knockdown of GluN2B or prior spinal administration of an antagonist selective for GluN2B-containing NMDARs impairs pain behavior in the second phase of the formalin test [143, 145, 146]. GluN2B antagonists also attenuate development of central sensitization and mechanical but not thermal hyperalgesia after spinal nerve ligation [144]. Contradictory to the observed NMDAR-independence of development of mechanical hyperalgesia after plantar incision, pretreatment with a GluN2B-selective antagonist reduces mechanical hyperalgesia in the same model [216]. By contrast, GluN2A may not be required for the development of formalin-induced pain behavior or hyperalgesia in inflammatory, neuropathic, or postoperative pain models [148, 216, 219]. The selective dependency on GluN2B- versus GluN2A-containing receptors in pain hypersensitivity is consistent with the enrichment of GluN2B and sparseness of GluN2A at synapses in the superficial DH and the reverse expression pattern of these subunits in deeper laminae [62]. Spinal GluN2C-containing receptors may contribute to the development of nocifensive behavior in the second phase of the formalin test [219].

Spinal Ca^{2+}-permeable AMPARs have been implicated in the induction of central sensitization and hyperalgesia in some pain models. Mechanical allodynia induced by burn injury and mechanical and thermal hyperalgesia induced by hindpaw carrageenan injection are prevented by intrathecal antagonists of Ca^{2+}-permeable AMPARs, whereas such antagonists do not affect the late phase of the formalin test or mechanical allodynia induced by spinal nerve ligation [136, 220, 221].

It has been suggested that spinal KARs also contribute to the induction of central sensitization and pain hypersensitivity. Spinal administration of SYM 2081, a KAR agonist that rapidly desensitizes the receptor and thereby functions as a de facto antagonist of such receptors, attenuates the development of capsaicin-induced mechanical and thermal hyperalgesia [222]. This appears in line with the observed role of postsynaptic GluK2-containing KARs in primary afferent LTP and in mediating Ca^{2+} influx in lamina II neurons [111] (see above). However, mice deficient in GluK1 but not those lacking GluK2 exhibit diminished nociceptive behavior after capsaicin injection and during all

phases of the formalin test, although whether this is attributed to an effect on KARs in the spinal cord or elsewhere is unknown [151]. It is possible that a reduction in KAR-mediated presynaptic inhibition of glutamate release from nociceptive primary afferent fibers (see above) could underlie the antinociceptive effect of SYM 2081.

Signaling Pathways

The development of secondary hyperalgesia and/or central sensitization is dependent on several different protein kinases, including CaMKII, PKC, PKA, extracellular signal-regulated kinase 2 (ERK2) and Src (e.g. [3, 223–229]; see Fig. 4). However, the extent to which different signaling molecules are involved vary between pain models. For example, while activation and/or autophosphorylation of CaMKII in the spinal DH has been shown to be involved in a number inflammatory, neuropathic and postoperative pain models [223, 224, 227, 230–235], CaMKII may be dispensable for the development of mechanical hypersensitivity associated with thermal injury [225]; moreover, autophosphorylation at Thr286 of CaMKIIα, which is the isoform that has been most extensively studied in conjunction with synaptic plasticity [205], may not be required for inflammatory or neuropathic hyperalgesia [236].

At hippocampal glutamatergic synapses, LTP induction involves translocation of dendritic CaMKII from cytosol to the PSD [205, 237–239]. This postsynaptic accumulation is accompanied by increased autophosphorylation of the kinase at the individual synapse and is thought to be a crucial step in the induction of LTP by promoting synaptic recruitment and phosphorylation of GluA1-containing AMPARs [205, 239, 240]. If LTP-like potentiation of nociceptive C fiber synapses occurs in models of hyperalgesia and central sensitization, it is therefore likely that CaMKII and pCaMKII translocate to the PSD at C fiber synapses in such models. Indeed, using immmunoelectron microscopy, it was demonstrated that both CaMKII and pCaMKII accumulate in the PSD of synapses established by peptidergic nociceptive primary afferent fibers within

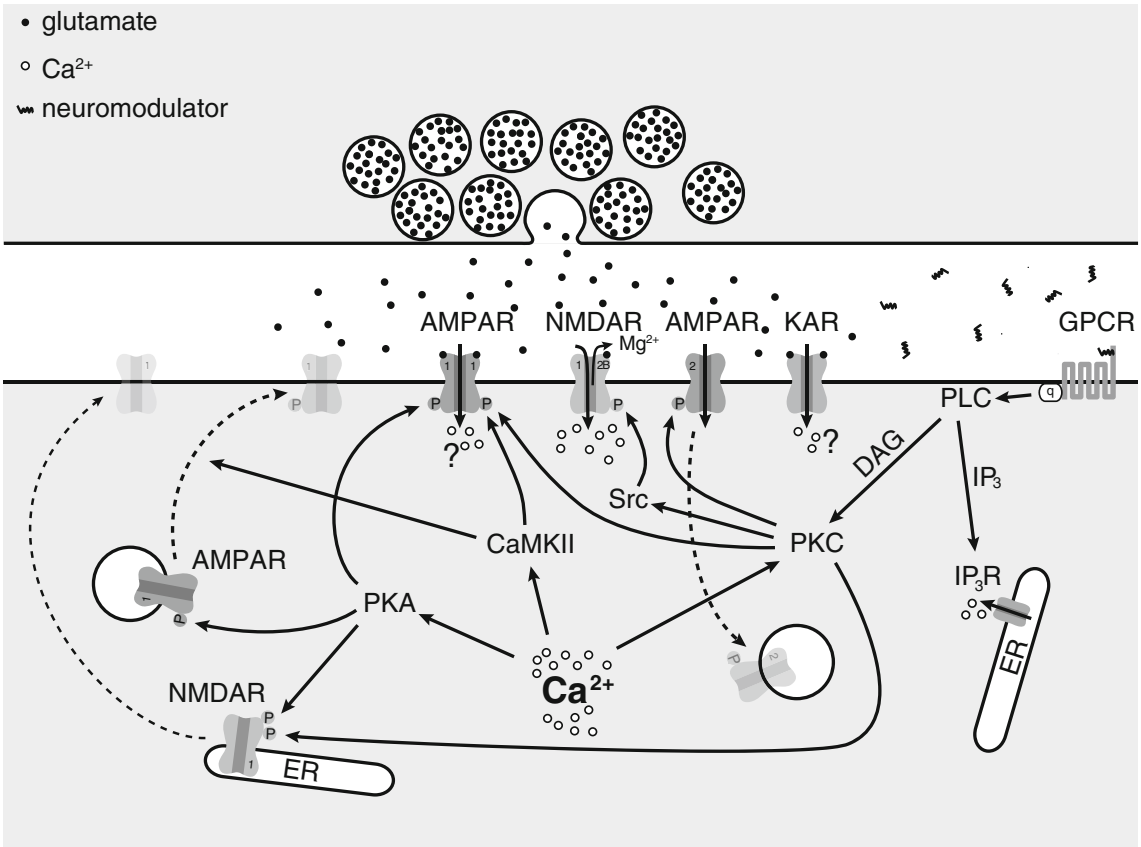

Fig. 4 Possible synaptic mechanisms in central sensitization and/or hyperalgesia. Release of glutamate and neuromodulators from their central terminals in the dorsal horn following intense noxious stimulation leads to Ca^{2+} influx into the postsynaptic dendrite through activated Ca^{2+}-permeable iGluRs and, for example via G_q-coupled receptors, release of Ca^{2+} from intracellular stores. Through different signaling cascades phosphorylation of NMDARs and AMPARs occurs, leading to modulation of receptor properties. NMDARs and GluA1-containing AMPARs are recruited to the synapse, whereas GluA2-containing AMPARs are internalized. Within the depicted iGluR subunits, *1* denotes GluA1 or GluN1, *2* denotes GluA2, and *2B* denotes GluN2B. *DAG* diacylglycerol, *ER* endoplasmatic reticulum, *GPCR* G-protein-coupled receptor, *P* phosphate group, *q* G_q protein. For other abbreviations, see text

20 min of intradermal capsaicin stimulation, suggesting that such synapses are indeed potentiated in this model of inflammatory pain hypersensitivity [231]. Curiously, however, whereas PSDs postsynaptic to nonpeptidergic C fibers contain high levels of pCaMKII in the basal state [241], cutaneous capsaicin stimulation leads to reduced PSD levels of CaMKII and pCaMKII at those synapses [231]; this reduction appears to occur preferentially at the cytosolic face of the PSD, perhaps indicating that CaMKII bound to postsynaptic membrane proteins such as GluN2B is less susceptible to this process.

Expression Mechanisms

NMDARs

General or GluN2B-selective NMDAR antagonists applied spinally after establishment of hyperalgesia and central sensitization in models of neuropathic or inflammatory pain often reverse or attenuate these phenomena, indicating that NMDARs contribute to their expression or maintenance [3, 142, 145, 146]. The reversal of pain hypersensitivity induced by NMDAR antagonism is sometimes observed to be transient, suggesting in these cases an involvement of NMDARs in expression rather than maintenance [142]. However, some forms of hyperalgesia or central sensitization are resistant to postinjury administration of NMDAR antagonists [129, 142, 242, 243].

Most studies have failed to detect changes in the spinal expression of GluN1, or its splice variants, at the mRNA or protein level in a number of different acute and chronic pain models [244–250]. However, the evidence is somewhat inconsistent; in the chronic constriction injury model of neuropathic pain, GluN1 protein or mRNA levels in the DH have been reported to either increase [251] or decrease [252, 253], while after spinal nerve ligation, no change was observed. GluN2 subunits show distinct patterns of expression in different pain models. Six hours after hindpaw formalin injection, GluN2A and GluN2C mRNA are increased and decreased, respectively, in lumbar spinal cord, while GluN2B and GluN2D expression are unchanged [246]. In the hindpaw carrageenan injection model of inflammatory pain, a reduction in spinal GluN2B protein levels was observed 1–5 days after carrageenan injection, while those of GluN2A did not change [245]. By contrast, after chronic constriction injury, GluN2B (but not GluN2A) is instead increased in laminae II–III [253], while no changes in the expression of any of the GluN2 subunits are evident in the spinal nerve ligation model [97, 248].

In DRG small- and medium-diameter neurons as well as in trigeminal ganglia, a reduction in GluN1 immunoreactivity is observed after peripheral complete Freund's adjuvant (CFA)-induced inflammation [254, 255]. Furthermore, the proportion of GluN1-possessing unmyelinated axons in peripheral nerve branches is transiently increased during inflammation, suggesting that the observed somatic reduction in GluN1 may be caused by an accelerated anterograde transport of NMDARs to peripheral terminations [256, 257]; however, whether there are similar changes in the amount of presynaptic NMDARs at central terminals of primary afferent fibers is unknown.

There are conflicting observations as to whether the NMDAR-mediated component of primary afferent synaptic transmission is upregulated in different pain models. Responses to NMDA are enhanced in spinothalamic tract neurons in the capsaicin model of acute inflammatory pain in primates [258] and in rat lamina II neurons in after spinal nerve ligation [259]. In line with this, a recent study reported, after induction of hindpaw inflammation by intraplantar injection of CFA, increased levels of GluN1 and GluN2B, but not GluN2A (see, however, [114]), in PSD-enriched subcellular fractions from lumbar DH ipsilateral to the inflamed skin; conversely, GluN1 was concomitantly decreased in crude synaptosomal fractions ipsilaterally [249]. The increase in synaptic GluN1, the time course of which paralleled the development of mechanical hyperalgesia, was dependent on NMDARs and PKA. However, the NMDAR-mediated component of primary afferent transmission onto lamina I $NK1R^{+}$ neurons is reduced at positive membrane potentials in spinal cord slices prepared 3 days after hindpaw CFA injection [107]. The Mg^{2+} sensitivity of NMDARs and potency of a GluN2B-selective antagonist are also reduced, indicating a decreased expression of GluN2B-containing receptors at these synapses [107]. A PKC-dependent reduction in voltage-dependence of NMDARs in dissociated DH neurons after CFA-induced inflammation has also been observed [260].

Enhanced PKC- and PKA-dependent phosphorylation of Ser896 and Ser897, respectively, in the C1 cassette of GluN1 have been observed in DH neurons in various models of pain hypersensitivity [244, 248, 250, 261–263]. Phosphorylation of these sites suppresses retention in the endoplasmatic reticulum and promotes increased NMDAR surface expression [264].

Tyrosine phosphorylation of GluN2B by Src kinases is increased in the DH in various inflammatory pain and neuropathic models. This phosphorylation depends on PKC, IP_3Rs, G_q-coupled GPCRs, phosphatidylinositol 3-kinase, and EphB receptor tyrosine kinase [226, 265–268] (however, see Caudle et al. [245]). Tyrosine phosphorylation of GluN2B results in inhibited endocytosis or increased gating of NMDARs [264, 269]. In further support of this, GluN2B tyrosine phosphorylation and hyperalgesia in inflammatory and neuropathic pain models is abolished by disruption of the coupling of Src to the NMDAR complex [226]. Ser1303 in GluN2B, a PKC/CaMKII

phosphorylation site of unclear significance but which has been implicated in potentiation of single-channel conductance [264], shows increased phosphorylation in the spinal cord in capsaicin-induced inflammatory pain [263].

Thus, most evidence so far indicate that the NMDAR-mediated component of synaptic transmission is increased in the spinal DH in different models of pain hypersensitivity and central sensitization. This may be attributed to recruitment of NMDARs, perhaps in particular such containing GluN2B, to synaptic and/or extrasynaptic sites, but also to reduced Mg^{2+} sensitivity or modulation of single-channel properties. However, the fact that NMDARs may be involved only in a subset of models of pain hypersensitivity could mean that this class of iGluR plays distinct roles at different sets of synapse within the neuronal circuitry of the DH. With the exception of the study by Vikman et al. [107], the regulation of different NMDAR subunits at identified spinal synaptic populations in different pain models is essentially unknown.

AMPARs

Inhibition of spinal AMPARs reversibly abolishes already established secondary hyperalgesia and sensitization of DH neurons in various inflammatory, neuropathic, postoperative and burn injury models of pain hypersensitivity [134, 159, 217, 220, 243, 270–275]. Interestingly, pain hypersensitivity in postoperative pain, which is essentially insensitive to NMDAR antagonism, is transiently reversed by posttreatment spinal administration of antagonists of Ca^{2+}-permeable AMPARs [271]. By contrast, the NMDAR-dependent pain hypersensitivity that accompanies subcutaneous formalin injection and spinal nerve ligation is insensitive to postinjury blockade of Ca^{2+} permeable AMPARs [136].

As is the case with NMDARs, the regulation of AMPAR subunit expression in the spinal cord varies between different pain models. The first report of a change in spinal AMPAR expression in a pain model was that of Zukin and co-workers, who showed a bilateral decrease in GluA1, but not GluA2 or GluA3, mRNA levels in both dorsal and ventral horns 24 h after induction of monoarthritis [247]. In capsaicin and CFA models of inflammatory pain, changes in mRNA or protein levels of GluA1 or GluA2 in the DH have generally not been observed [276–279]. However, one study observed a spinal upregulation of mRNA encoding some flip/flop variants of GluA1-3, as well as increases in GluA1 and GluA2 protein in the superficial DH, within the first 24 h after hindpaw CFA injection [280]. After formalin injection in the whisker pad of rats, the number of cells immunoreactive for GluA1 and GluA2/3 was found to be reduced in the superficial medullary DH [281]. In the spinal nerve ligation and chronic constriction injury models of neuropathic pain, spinal upregulation of GluA1-3 subunits in the DH has been reported [223, 282–284] (but see [277]), while spinal nerve transection was observed to result in enhanced transcription of GluA3 and GluA4 in the dorsal spinal cord [285].

Primate spinothalamic tract neurons exhibit enhanced responses to AMPAR agonists after intradermal capsaicin injection [258]. In rats, after hindpaw carrageenan injection, a more prolonged model of inflammatory pain, potentiated EPSCs and Ca^{2+} transients in response to AMPA were evident, although only during inhibition of AMPAR desensitization [286]. Hindpaw injection of capsaicin or formalin induces LTP of C fiber-evoked field potentials in lamina II in intact rats; although not directly tested, this LTP is likely attributed to potentiation of the AMPAR component of C fiber synaptic transmission [178]. Within 20 min after cutaneous capsaicin stimulation in rats, GluA1-containing AMPARs are translocated to some nociceptive primary afferent synapses whereas GluA2/3 levels are unchanged at the same synapses, providing further support for a potentiation of AMPAR-mediated primary afferent synaptic transmission in this model of acute inflammatory pain [79]. In vivo patch-clamp recordings of lamina II neurons in adult rats 24 h after hindpaw CFA injection revealed that neurons with input from inflamed skin had more frequent large-amplitude spontaneous AMPA EPSCs compared to neurons lacking such input [114]. By contrast, 1 or 3 days after hindpaw CFA injection, no change was detected in the amplitude of AMPAR-mediated EPSCs at primary afferent synapses with lamina I $NK1R^+$ neurons or lamina II neurons in vitro [107, 278].

Mice deficient in the GluA2 subunit exhibit enhanced mechanical and thermal hyperalgesia after hindpaw CFA injection and exaggerated pain behavior throughout the formalin test [113], as well as an increased susceptibility and NMDAR independence of primary afferent LTP [197]. Thus, it is likely that Ca^{2+}-permeable AMPARs, if upregulated in DH synapses, may have a profound effect on central sensitization and hyperalgesia. In fact, there is evidence of an increased involvement of Ca^{2+}-permeable AMPARs in spinal sensory transmission in acute and chronic models of inflammatory hyperalgesia. For example, primary afferent fiber-evoked AMPA-mediated EPSCs show increased inward rectification and are more sensitive to antagonists selective for Ca^{2+}-permeable AMPARS in lamina II neurons [114, 278] and in lamina I $NK1R^+$ neurons [107] after hindpaw CFA injection.

Phosphorylation of GluA1 at the PKC/CaMKII site Ser831, which increases single-channel conductance of homomeric GluA1-containing receptors [287], is enhanced in the dorsal horn after intradermal capsaicin [276, 288], after plantar incision [224] and after hindpaw CFA injection [277]. By contrast, no enhanced phosphorylation at Ser831

was detected by Western blot in the spinal cord after burn injury [225] or spinal nerve ligation [277]. Inhibiting either PKC or CaMKII abolishes phosphorylation of this site in the DH after intradermal capsaicin injection [230, 288]; which of these kinases phosphorylates the site directly is unclear. As for Ser831, the phosphorylation level of the PKA site Ser845 in GluA1 is increased in the DH after hindpaw capsaicin or CFA injection [63, 276, 277, 288]; specifically, a distinct increase in phosphorylation of Ser845 occurs at a subset of postsynaptic sites in the medial part of laminae I–II after capsaicin stimulation [63]. The enhanced phosphorylation of GluA1 at both Ser831 and Ser845 that occurs in the DH after hindpaw CFA injection abates within 12 h, suggesting that such phosphorylation is selectively involved in the early phase of the model [277].

Some recent evidence suggest that GluA2-containing receptors may be internalized at primary afferent synapses in CFA-induced inflammatory hyperalgesia [114, 278]. If endocytosed GluA2-containing AMPARs are replaced by Ca^{2+}-permeable AMPARs at these synapses, it would be expected that primary afferent-evoked postsynaptic Ca^{2+} transients are enhanced. This could in turn lead to increased activation of Ca^{2+}-dependent processes that promote increased membrane excitability, for example via phosphorylation of $K_V4.2$ channels [172]. Phosphorylation of Ser880 in the GluA2 subunit rapidly becomes elevated in the DH after hindpaw CFA injection and stays at elevated levels for several days [278]. This leads to dissociation of GluA2 from glutamate receptor interacting protein (GRIP), and subsequent internalization of the GluA2-containing receptors from the synapse (see below). Another protein implicated in synapse retention of GluA2-containing AMPARs, *N*-ethylmaleimide-sensitive fusion protein (NSF), is downregulated in the PSD in the spinal cord after hindpaw CFA injection, further supporting removal of such receptors from spinal synapses in this pain model [114]. Transgenic mice expressing mutant GluA2 unable to phosphorylate at Ser880 exhibit intact CFA-induced hyperalgesia 2 h after injection but reduced hyperalgesia after 1 day onwards, indicating that internalization of GluA2-containing AMPARs contributes to the late but not the early phase of this model of inflammatory hyperalgesia [278].

Recruitment of GluA1-containing AMPARs to synapses is thought to be a major mechanism contributing to synaptic potentiation in various parts of the nervous system [54]. Such trafficking of GluA1-containing receptors has been proposed to also underlie potentiation of primary afferent synapses in hyperalgesia. It was initially reported that plasma membrane levels of GluA1 rapidly increase in a CaMKII-dependent manner in the spinal cord after instillation of capsaicin in the colon [233]. Furthermore, a postsynaptic increase in GluA1 levels is observed at C fiber synapses 20 min after hindpaw capsaicin injection [79] (see below). Because phosphorylation by PKA of Ser845 in GluA1 is essential for surface expression of AMPARs containing this subunit, the increased phosphorylation of this site observed after hindpaw capsaicin injection [63, 276, 288] supports translocation of GluA1-containing AMPARs to DH synapses in this pain model. Increased plasma membrane or PSD levels of GluA1 have been observed in the DH after hindpaw CFA [279, 289] or formalin [268] injection. However, the time course of GluA1 trafficking in the CFA model is considerably slower than in more acute pain models [279].

It has been proposed that synapses formed by peptidergic primary afferent fibers onto lamina I neurons are involved in inflammatory hyperalgesia, whereas synapses formed by nociceptive C fibers that lack such neuropeptides have been implicated in neuropathic pain. The latter hypothesis has been primarily based on the assumption that nonpeptidergic C fibers establish connections with neurons in lamina IIi that express PKCγ, which is essential for neuropathic hyperalgesia. However, this assumption has recently been shown to be incorrect, as such neurons selectively receive low-threshold primary afferent input [40]. Furthermore, the methods most commonly used to study the molecular mechanisms of central sensitization lack the ability to differentiate between different C fiber synaptic populations in the superficial DH. Thus, whether peptidergic and nonpeptidergic nociceptor pathways have distinct roles in different forms of nociception and pain hypersensitivity remains largely unknown. To address this problem, we used transganglionic tracing and postembedding immunogold labeling to examine at the ultrastructural level whether different populations of primary afferent synapse undergo molecular changes indicative of synaptic plasticity in the capsaicin model of acute inflammatory hyperalgesia. Using this experimental strategy, we found that the GluA1 subunit rapidly accumulates at synapses established by nonpeptidergic C fibers that innervate capsaicin-stimulated skin; by contrast, the levels of GluA2/3 at such synapses are only subtly increased [79]. Thus, nonpeptidergic C fiber synapses in the DH may be potentiated in this pain model by recruiting GluA1-containing, possibly Ca^{2+}-permeable AMPARs. However, although synaptic trafficking of GluA1-containing AMPARs is thought to rely on the activation of CaMKII, both CaMKII and pCaMKII are downregulated in the PSD of these synapses. One possible solution to this apparent paradox may be that an extrasynaptic pool of CaMKII, rather than CaMKII within the PSD, is responsible for the synaptic recruitment of GluA1-containing AMPARs. Nonpeptidergic C fibers form no or few monosynaptic connections with spinal projection neurons, but postsynaptic targets probably include both excitatory vertical cells and inhibitory islet cells in lamina II, among others (Fig. 1).

Because it is unknown whether nonpeptidergic C fiber synapses with excitatory or inhibitory neurons, or both, are potentiated after capsaicin stimulation, it is also unclear whether such potentiation contribute to facilitation or inhibition of spinal nociceptive transmission in this model.

Little or no change in the levels of GluA1 is observed at lamina I synapses formed by peptidergic nociceptors 20 min after capsaicin stimulation [79]. However, this does not necessarily indicate that these synapses are not potentiated. Both CaMKII and pCaMKII are upregulated at such synapses [231], suggesting the possibility that CaMKII-mediated phosphorylation of GluA1 at Ser831, which may enhance single-channel conductance of the receptor, could result in synaptic potentiation of peptidergic nociceptive synapses after capsaicin injection.

KARs

Relatively little is known about the putative role for KARs in the expression or maintenance of central sensitization. After carrageenan-induced hindpaw inflammation, the inhibitory effect of a spinally administered GluK1-selective KAR antagonist on the C fiber-evoked responses of deep DH neurons is enhanced, suggesting an increased contribution of GluK1-containing KARs to such responses in central sensitization [159]. Furthermore, in primate spinothalamic tract neurons, the increased or aberrant responses to mechanical and thermal stimuli in neuropathic animals were found to be more sensitive to a GluK1-selective antagonist as compared to normal responses in non-injured animals [125]. Intrathecally applied KAR antagonists have also been observed to reverse thermal hyperalgesia after hindpaw CFA injection; however, because thermal sensitivity in non-inflamed hindpaws was also decreased by the doses of antagonist used, it was difficult to distinguish an anti-hyperalgesic effect from a purely analgesic effect of such KAR blockade [130]. Nevertheless, the same study showed increased mRNA levels of GluK1 and unedited GluK2 in the dorsal spinal cord after hindpaw CFA, suggesting an increased contribution of postsynaptic KARs to spinal sensory transmission in this model of inflammatory pain [130].

iGluR-Interacting Proteins

A number of studies have implicated scaffolding and other proteins associated with iGluRs in central sensitization or hyperalgesia. The membrane-associated guanylate kinase (MAGUK) proteins are a family of scaffolding proteins that contain three consecutive N-terminal PDZ (postsynaptic density protein 95 (PSD-95)/Disks large/Zonula occludens-1) domains, a Src homology 3 (SH3) domain and a C-terminal guanylate kinase-like domain [290, 291]. PSD-95 and PSD-93 are two closely related MAGUK proteins that bind to GluN2 subunits of the NMDAR and are involved in synaptic AMPAR trafficking. PSD-95, the most abundant MAGUK protein in the PSD of central synapses, is found at synaptic sites throughout the gray matter of the spinal cord, but is particularly prominent in the superficial DH [64]. Spinal knockdown of PSD-95 impairs the development and maintenance of mechanical and thermal hyperalgesia after spinal nerve transection, while not affecting acute nociception or locomotor function [292, 293]. Furthermore, transgenic mice expressing a truncated version of PSD-95 lacking the third PDZ domain and the SH3 and guanylate kinase domains do not develop thermal or mechanical hyperalgesia after chronic constriction injury but exhibit normal formalin-induced nocifensive behavior [223]. The truncated PSD-95 does not localize to PSDs, and while in principle it can bind GluN2B, it is not immunoprecipitated with NMDARs, which show normal synaptic localization in the hippocampus [294]; thus, although a truncated PSD-95 is expressed in these mice, the synaptic functional repertoire of the protein is, presumably, completely disrupted. In the spinal cord of PSD-95 mutant mice, the amount of CaMKIIα bound to GluN2A/B-containing receptors is severely reduced, and neither constitutive CaMKII activity nor the amounts of CaMKIIα or pCaMKII bound to GluN2A/B are enhanced after nerve injury as in wild-type mice [223]. A Tat fusion peptide containing the second PDZ domain of PSD-95 reduces the interaction of PSD-95 with GluN2B and when given intrathecally (or systemically) attenuates both the development and maintenance phases of mechanical hypersensitivity after CFA-induced hindpaw inflammation [295]. A small-molecule inhibitor and a Tat fusion peptide that both interfere with the interaction between PSD-95 and neuronal nitric oxide synthase (nNOS) were recently shown to reduce mechanical hyperalgesia after chronic constriction injury, suggesting that localization of nNOS to the NMDAR complex within the PSD is essential for central sensitization in this pain model [296]. Like PSD-95, PSD-93 is enriched in the superficial DH [297]. Mice lacking PSD-93 exhibit abolished or much reduced mechanical hypersensitivity after hindpaw CFA injection or spinal nerve transection, whereas acute mechanical and thermal nociception are unaffected; cultured DH neurons from such mice also show reduced surface expression of GluN2A and GluN2B, and dorsal-root-evoked EPSPs have a reduced NMDAR component but an intact AMPAR component [297]. Furthermore, spinal knockdown of PSD-93 was observed to attenuate and postpone the development of mechanical and thermal hyperalgesia after hindpaw CFA injection and spinal nerve transection, respectively [298]. SAP97, which is the only MAGUK protein that directly binds GluA1, has been implicated in

CaMKII-mediated synaptic trafficking of GluA1-containing AMPARs [290, 299]. SAP97 is expressed in the spinal DH [300], but its role in pain hypersensitivity or in synaptic function in the spinal cord has not been investigated. Another MAGUK protein, SAP-102, which at hippocampal synapses (but not in whole hippocampus) is more abundant during embryonic and early postnatal development than in the adult animal [301], is expressed in the spinal cord of adult rats [302], although the precise cellular and subcellular distribution of the protein within spinal gray matter is unknown.

The C-terminal tail of the GluA2 subunit of AMPARs binds to a variety of scaffolding and adaptor proteins that are involved in AMPAR trafficking , including NSF, GRIP, AMPAR-binding protein (ABP; also called GRIP2), and protein interacting C kinase 1 (PICK1) [53, 55, 303]. The GluA2–NSF interaction and the ATPase activity of NSF are both required for retention of GluA2-containing receptors at the synapse. Similarly, it is thought that binding of GRIP to the PDZ binding sequence of GluA2 helps maintain the receptor in the synaptic membrane. Phosphorylation of GluA2 at Ser880 by PKC (or at Tyr876 by Src) disrupts the GluA2-GRIP interaction but allows binding of the same motif by PICK1, inducing endocytosis of the receptor. This sequence of events is thought to contribute to LTD at some synapses. The first report of a role for GluA2-binding proteins in synaptic plasticity in the spinal DH was a study by Zhuo and co-workers, who showed that in spinal slices from postnatal rats, serotonin-induced recruitment of AMPARs to previously silent DH synapses depends on the interaction between GluA2 and the PDZ domain of GRIP/ABP [304]. Because silent synapses are very rare in the mature superficial DH [78], and because GluA2-containing AMPARs have not been observed to be recruited to spinal synapses in models of hyperalgesia or central sensitization, the relevance of this mechanism to acute or chronic pain in adult animals is unclear. However, both GRIP/ABP and GluA2 expression is increased in the superficial DH following chronic constriction injury [284]. Disrupting the interaction of GluA2 with GRIP/PICK1 in the spinal cord using a GluA2-derived peptide that recognizes the PDZ domains of both GRIP and PICK1 attenuates thermal but not mechanical hyperalgesia after nerve injury [284]. A peptide that only interferes with GluA2-PICK1 binding also reduces thermal hyperalgesia to a similar degree, indicating that this interaction may be more important than that between GluA2 and GRIP. This suggests that endocytosis of GluA2-containing receptors may contribute to hyperalgesia in this model of neuropathic pain. Likewise, disruption of the GluA2-GRIP/ABP interaction by PKC-mediated phosphorylation of Ser880 in GluA2, as well as internalization of GluA2-containing AMPARs, accompany pain hypersensitivity after hindpaw CFA injection; moreover, mice carrying a K882A point mutation in GluA2 that prohibits phosphorylation of Ser880 exhibit an attenuated maintenance phase of CFA-induced hyperalgesia [278]. The PSD pool of NSF, which is implicated in synaptic anchoring of GluA2-containing AMPARs, is reduced in the spinal cord after hindpaw CFA injection [114], and NSF expression is downregulated in laminae I–II after chronic constriction injury [284], suggesting an involvement of this protein in modulation of spinal sensory transmission in these pain models.

A family of transmembrane proteins that closely associate with AMPARs (and may be considered auxiliary AMPAR subunits) is the transmembrane AMPAR regulatory protein (TARP) family, which includes γ2 (the first TARP identified, also called stargazin from the stargazer mutant mouse that lacks a functional version of this protein), γ3, γ4, and γ8 [305–307]. Two other more distant homologs, γ5 and γ7, have recently been suggested to constitute a type II family of TARPs [308]. Most TARPs are crucial for AMPAR trafficking by mediating transport of the AMPAR/TARP complex from the endoplasmatic reticulum to extrasynaptic plasma membrane. Moreover, lateral diffusion of the AMPAR/TARP complex into the synapse enables interaction of TARP with PSD-95, which anchors the receptor in the postsynaptic membrane. However, the relation between TARP-mediated trafficking of AMPARs and the subunit-specific trafficking of AMPARs that depends on the C-terminal tail of the different AMPAR subunits is unclear [309]. In addition to their role in receptor trafficking, TARPs strongly influence biophysical and pharmacological properties of AMPARs [305–307]. Knockdown of the TARP γ2 was observed to reduce the second, but not the first, phase of formalin-induced nocifensive behavior, while acute nociception and locomotor function were unaffected [310]. While other TARPs, including γ4 and γ5, are also expressed in the superficial DH according to the Allen Spinal Cord Atlas [300], their role in pain or spinal synaptic function has not yet been investigated. Another family of AMPAR auxiliary subunits that affect receptor surface expression and kinetics, the proteins cornichon homolog 2 (CNIH-2) and 3 (CNIH-3), has recently been identified [311]. These proteins may be localized to subpopulations of AMPARs partly distinct from those that contain TARPs, but their expression in the spinal cord is unknown. Moreover, a transmembrane protein, neuropilin tolloid-like 2 (Neto2) has been demonstrated to be associated with KARs and affect their functional properties but not surface trafficking of such receptors [312]. A related protein, Neto1, has been reported to associate with NMDARs and affect synaptic but not surface levels of GluN2A-containing NMDARs [313]; however, when expressed heterologously with the KAR subunit GluK2, Neto1 enhances glutamate-evoked currents of such homomeric KARs [312]. Both Neto1 and Neto2 are expressed in

the superficial DH [300], suggesting that they may be involved in spinal nociceptive transmission.

Conclusions

Now, 50 years on, the specificity of glutamate's action on DH neurons doubted by Curtis, Phillis, and Watkins is indisputable and explained by the ubiquitous presence of a variety of glutamate receptors at primary afferent and intrinsic excitatory synapses on every neuron in the DH. It is also clear that regulation by and of glutamate receptors underlies plastic changes in spinal sensory pathways and thereby contributes to central sensitization and pain hypersensitivity. Whereas it is now relatively apparent that mechanisms similar to LTP may occur at nociceptive C fiber synapses in NMDAR-dependent pain hypersensitivity, our understanding of the molecular mechanisms and physiological function of spinal synaptic plasticity in different pain models remains incomplete. For example, what synapses in the spinal sensory circuitry may be subject to such plasticity? To what extent does spinal LTP rely on trafficking of iGluRs to or from the synapse versus modulation of the functional properties of receptors already at the synapse? What determines the distinct molecular dependencies exhibited by different acute and chronic pain models? Addressing these questions may not only provide clues to future targets for the treatment of pathological pain but may also improve our general understanding of synaptic plasticity and experience-dependent modification of synaptic circuitry.

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