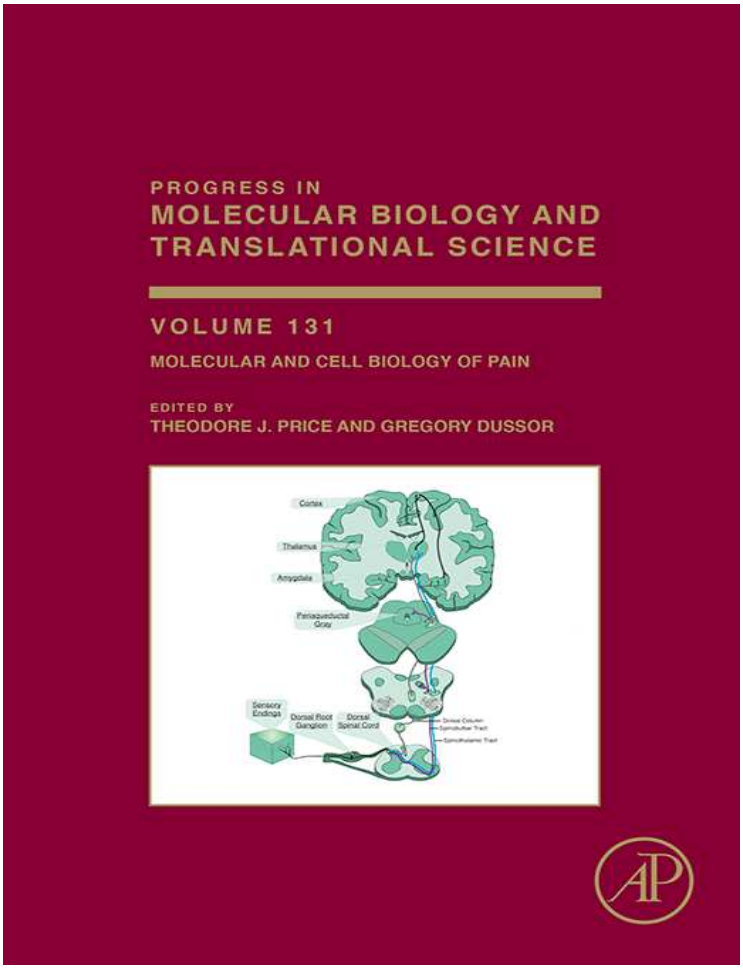

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CHAPTER TEN

mGluRs Head to Toe in Pain

Benedict J. Kolber[1]
Department of Biological Sciences, Duquesne University, Pittsburgh, Pennsylvania, USA
[1]Corresponding author: e-mail address: kolberb@duq.edu

Contents

Abstract

Metabotropic glutamate receptors (mGluRs) and their role in modulating pain throughout the peripheral and central nervous system are the focus of this chapter. Because these receptors are so prolifically involved in pain signaling throughout the neuraxis, we will use them as a vehicle to explore the totality of the neuraxis. These diverse receptors can increase or decrease pain depending on the subtype of receptor involved and anatomical location of activity. We will cover the basic molecular structure and function of mGluRs and then evaluate the role of different mGluRs at each level of the pain neuraxis. Similar to the functional anatomy involved in the processing of exogenous noxious stimuli, we will start from the peripheral nociceptive terminal and end in higher brain centers that are involved in the cognitive and emotional components of pain. We will conclude by examining the cutting-edge technology involved in the development of mGluR agents for the treatment of pain.

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1. INTRODUCTION

Since pain is subjective, understating pain in nonhumans depends on behavioral changes (which themselves may be mediated through neurons expressing and responding to glutamate through mGluRs) or on measurement of the underlying phenomena (EPSPs, IPSPs) thought to underlie the neuronal communication of noxious stimuli. Experimentally, we induce pain by selective application of inflammatory agents (e.g., formalin) or injury to some part of the peripheral (e.g., sciatic nerve) or central nervous system (e.g., spinal cord). Behavioral output is measured by monitoring spontaneous behavior (e.g., lifting, licking, biting of affected area), evaluating social interactions and conditioning, or by monitoring responses to experimenter-delivered stimuli. These stimuli include mechanical von Frey filaments or heat/cold stimuli. mGluRs have been evaluated at each stop along the pain neuraxis.

These diverse receptors can increase or decrease pain depending on the subtype of receptor involved and anatomical location of activity. We will cover the basic molecular structure and function of mGluRs and then evaluate the role of different mGluRs at each level of the pain neuraxis. Similar to the functional anatomy involved in the processing of exogenous noxious stimuli, we will start from the peripheral nociceptive terminal and end in higher brain centers that are involved in the cognitive and emotional components of pain. We will conclude by examining the cutting-edge technology involved in the development of mGluR agents for the treatment of pain.

2. METABOTROPIC RECEPTORS

Metabotropic G-protein-coupled receptors (GPCRs) can mediate both quick and long-lasting signaling changes through interaction with G-proteins. G-proteins are a trimeric signaling complex that consist of alpha, beta, and gamma subunits. At baseline most G-proteins are inactive. An inactive G-protein is converted to an active form when bound GDP is exchanged for GTP in the alpha subunit of the G-protein. The G-protein then slowly hydrolyzes the GTP to GDP and then becomes inactive; in this way, the G-protein has a sort of ingenious internal timer. Depending on the type of receptor and ligand, activation of a G-protein can activate or inhibit cell-signaling cascades and, for excitable neurons,

can ultimately activate or inhibit the cell itself. GPCRs can be found on the soma, dendrites, or axons of neurons and can modulate glial function. The GPCRs are targets for roughly one-third of all medications, and they are prolifically involved in both acute and chronic pain. A better understanding of GPCRs and their role in pain may be the key to unlocking the mystery of the complex transition from acute to chronic pain. Well-studied GPCRs in pain include the opioid receptors, serotonin receptors, ATP receptors, neurokinin receptor, corticotropin-releasing factor receptors, glutamate receptors, and countless others. Given the prominence of glutamate at many nociceptive synapses, it is not surprising that G-protein-coupled glutamate receptors (better known as mGluRs) have received considerable attention from academic and industrial scientists.

2.1 Metabotropic glutamate receptors

mGluRs (synonyms: GRM#, mGluR#, mGlu#, where "#" stands for subtype number) are found throughout the peripheral and central nervous system where they fine-tune signaling through NMDA and AMPA ionotropic glutamate receptors (iGluRs).[1] mGluRs belong to class C GPCRs, which are characterized by a large N-terminal extracellular domain, seven transmembrane domains, and highly variable C-terminal intracellular domains (reviewed in Refs. 1,2) (Fig. 1). The N-terminal extracellular domains of three mGluRs have been crystalized.[3–5] These crystallized domains contain several important motifs for mGluR function; first, they contain the Venus flytrap domain, which is thought to be critical for ligand binding (i.e., glutamate), exogenous modulation (by orthostatic agonist/antagonists; Table 1), and constitutive receptor dimerization; second, they contain a cysteine-rich domain that propagates conformation changes due to ligand binding to the transmembrane, or heptahelical, domains.[2] The transmembrane domains are responsible for activation of the intracellular G-protein and are thought to contain binding sites for multiple allosteric modulators of mGluRs (Table 1).[2,6] The various cytoplasmic C-terminal domains of mGluRs contain binding sites for a variety of intracellular proteins, sites for posttranslational modification, and are a major area of splice variant expression.[7] Eight distinct mGluRs (mGluR1, mGluR2, mGluR3, mGluR4, mGluR5, mGluR6, mGluR7, mGluR8) belonging to three groups (Group I, Group II, Group III) have been identified based on sequence homology[1] (Fig. 1).

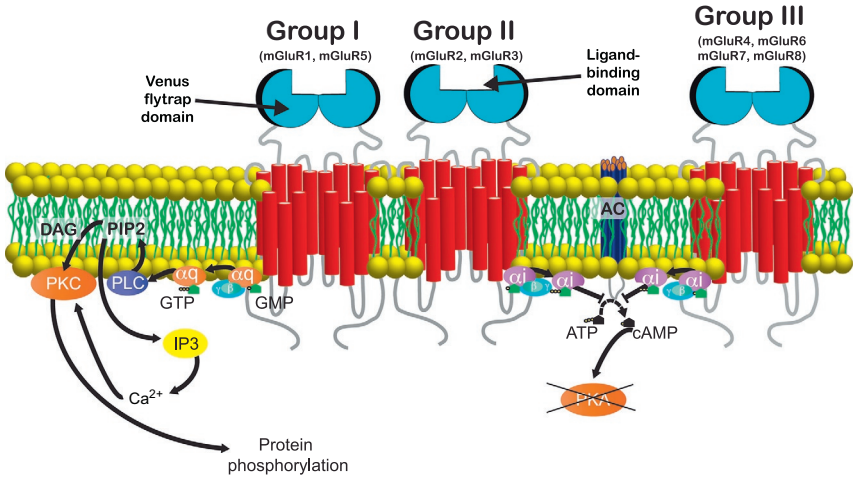


Figure 1 Schematic illustration of mGluR dimers from Group I, II, and III receptors. All mGluRs contain large extracellular domains known as Venus flytrap domains. These domains contain the binding site for glutamate and other agents. Group I mGluRs (mGluR1 and mGluR5) couple to the G-protein $G_{\alpha q}$. $G_{\alpha q}$ activates phospholipase C (PLC) which hydrolyzes phospholipid phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) to generate DAG and IP3 to increase intracellular calcium and activate PKC. Both Group II (mGluR2 and mGluR3) and Group III (mGluR4, mGluR6, mGluR7, mGluR8) mGluRs couple to the G-protein $G_{\alpha i}$. Activated $G_{\alpha i}$ inhibits the conversion of ATP to cAMP by adenylyl cyclase (AC), which ultimately lowers protein kinase A (PKA) activation.

2.1.1 Group I mGluRs (mGluR1 and mGluR5)

Group I mGluRs include mGluR1 and mGluR5. mGluR1 is associated with four main splice variants (mGluR1a, b, c, d) and mGluR5 with two variants (mGluR5a, b).[2] Group I mGluRs are most commonly associated with the G_q/G_{11} subtype of G-protein, which activates phospholipase C (PLC) (Fig. 1). PLC hydrolyzes phospholipid phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) to generate diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3). This pathway leads to increased intracellular calcium and activation of protein kinase C (PKC). A number of studies[2] including those studying the role of Group I mGluRs in pain[8,9] have shown that these receptors can modulate other downstream signaling cascades and G-proteins including $G_{i/o}$ and G_s. Group I mGluRs are associated with increased activation of the mitogen-activated protein kinase pathway,[10] protein kinase A (PKA),[8] potassium channel $Kv_{4.2}$,[10] and regulation by Homer proteins,[11] all of which are associated with amplification of pain signaling. These mGluRs (Group I) are expressed on neurons throughout the pain

Table 1 Targets and names of mGluR pharmacological agents listed in text

mGluR group	Target	Compound; chemical name	Activity
Nonspecific	Group I/II > Group III	(1*S*,3*R*)-ACPD; (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid	Antagonist
	Group II > Group III > Group I	LY341495; [2(*S*)-2-amino-2-(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-xanth-9-yl) propanoic acid	Antagonist
	mGluR1/5	(*S*)-3,5-DHPG; (*S*)-3,5-dihydroxyphenylglycine	Agonist
	mGluR1 > mGluR5	AIDA; 1-aminoindan-1,5-dicarboxylic acid	Antagonist
	mGluRI	LY367385; (*S*)-(+)-a-amino-4-carboxy-2-methylbenzeneacetic acid	Antagonist
Group I	mGluRI	4-CPG; (*S*)-4-carboxyphenylglycine	Antagonist
	mGluRI	CPCCPEt; 7-(hydroxyimino)cyclopropa[*b*]chromen-1a-carboxylate ethyl ester	NAM
	mGluR5	CHPG; (*RS*)-2-chloro-5-hydroxyphenylglycine	Agonist
	mGluR5	MPEP; 2-methyl-6-(phenylethynyl)pyridine	NAM
	mGluR5	MTEP; 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine	NAM
	mGluR5	Fenobam; 1-(3-chlorophenyl)-3-(3-methyl-5-oxo-4H-imidazol-2-yl) urea	NAM
	mGluR2/3	LY354740; (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid	Agonist
	mGluR2/3	DCG-IV; (2*S*,10*R*,20*R*,30*R*)-2-(20,30-dicarboxycyclopropyl) glycine	Agonist

Continued

Table 1 Targets and names of mGluR pharmacological agents listed in text—cont'd

mGluR group	Target	Compound; chemical name	Activity
Group II	mGluR2/3	L-CCG-I; (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine	Agonist
	mGluR2/3	APDC; (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate	Agonist
	mGluR2/3	LY404039; (−)-(1 *R*,4*S*,5*S*,6*S*)-4-amino-2-sulfonylbicyclo[3.1.0] hexane-4,6-dicarboxylic acid	Agonist
	mGluR2/3 > group III	EGLU; (2*S*)-alpha-ethylglutamic acid	Antagonist
	mGluR4,6,8 > mGluR7	LAP4; L-2-amino-4-phosphonobutyric acid	Agonist
	mGluR8 > mGluR4/6	DCPG; (*S*)-3,4-dicarboxyphenylglycine	Agonist
	Group III	L-SOP; L-serine-*O*-phosphate	Agonist
	Group III	MAP4; (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid	Antagonist
Group III	Group III	CPPG; (*RS*)-a-cyclopropyl-4-phosphonophenylglycine	Antagonist
	Group III	MSOP; (*RS*)-a-methylserine-*O*-phosphate	Antagonist
	Group III	UBP112; α-methyl-3-methyl-4-phosphonophenylglycine	Antagonist
	mGluR4	PHCCC; *N*-phenyl-7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxamide	PAM
	mGluR7	AMN082; *N*,*N*′-dibenzhydrylethane-1,2-diamine dihydrochloride	Agonist

This is a list of the main pharmacological agents that have been used to understand the role of mGluRs in pain and are referred to in the text. PAM, positive allosteric modulator; NAM, negative allosteric modulator.

neuraxis, including in the peripheral nociceptors (including DRG),[12–16] spinal cord neurons,[17,18] periaqueductal gray (PAG),[19] thalamus,[20] amygdala,[21] and prefrontal cortex (PFC)[22] (Fig. 2). Activation of Group I mGluRs usually, although not always, increases pain or pain-like responses.

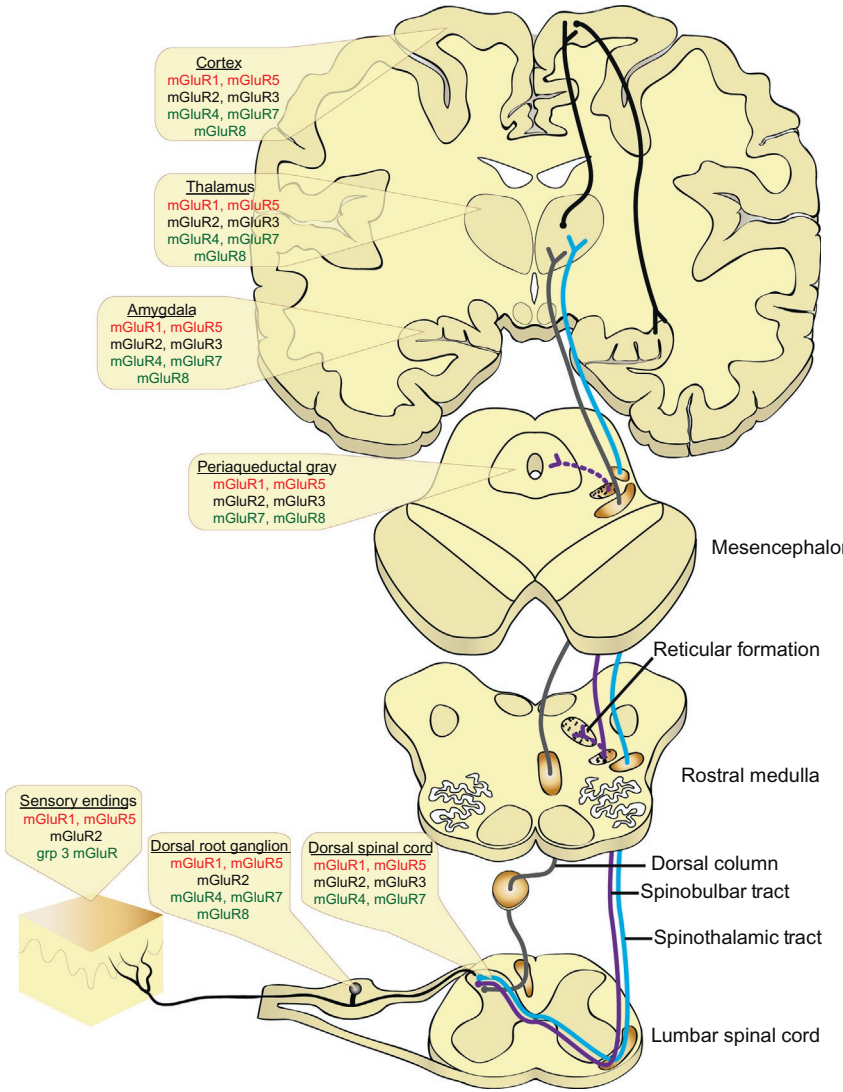


Figure 2 Illustration of mGluR expression at different levels of the pain neuraxis. mGluRs are expressed throughout the nervous system including at all major sites involved in nociception and pain. *Image adapted from original art by Maria Elena Morales and previous publication,[23] used with permission.*

2.1.2 Group II mGluRs (mGluR2 and mGluR3)

Group II mGluRs include mGluR2 and mGluR3. There are no known splice variants of mGluR2. mGluR3, by contrast, has four splice variants: the full-length protein, GRM3Δ2 (missing exon 2), GRM3Δ4 (missing exon 4), and GRM3Δ2Δ3 (missing exons 2 and 3).[24] Group II mGluRs are most commonly associated with the G_i/G_o G-protein, which inhibits adenylyl cyclase and PKA (Fig. 1). A number of studies including those studying the role of Group II mGluRs in pain have shown that these receptors can modulate other downstream signaling cascades to activate the mitogen-activated protein kinase pathway and PI3 kinase.[9] Similar to Group I mGluRs, Group II mGluRs are expressed throughout the pain neuraxis[17,20,25–28] (Fig. 2). Specific delineation of mGluR2 versus mGluR3 expression, however, has been hampered by nonspecificity of staining between the receptors in many antibody histochemical studies.[13,29] Activation of Group II mGluRs typically dampens nociceptive signaling.

2.1.3 Group III mGluRs (mGluR4, mGluR6, mGluR7, and mGluR8)

Group III mGluRs include mGluR4, mGluR6, mGluR7, and mGluR8. mGluR4 has one splice variant (taste mGluR4) that is found in taste neurons. mGluR6 and mGluR8 are each associated with three main splice variants (mGluR6a, mGluR6b, mGluR6c; mGluR8a, mGluR8b, mGluR8c).[30,31] mGluR7 is associated with five variants (mGluR7a, mGluR7b, mGluR7c, mGluR7d, mGluR7e).[32] Three Group III variants (mGluR6b, mGluR6c, mGluR8c) terminate within the N-terminus and may be soluble receptors for glutamate.[30,31] None of the minor Group III splice variants have been specifically studied in the context of pain. Like Group II mGluRs, Group III mGluRs are most commonly associated with the G_i/G_o, which inhibits adenylyl cyclase and PKA (Fig. 1). Similar to Group I and II mGluRs, Group III mGluRs are expressed throughout the pain neuraxis[13,18,33–35] (Fig. 2). Activation of Group III mGluRs typically dampens nociceptive signaling.

3. ANALYSIS OF mGluRs AT EACH LEVEL OF THE PAIN NEURAXIS

mGluRs, as indicated above, are expressed throughout the pain neuraxis on both presynaptic and postsynaptic neurons and on glial cells. In the following sections, the role of each mGluR group will be described at each individual section of the pain neuraxis. This organizational layout is meant

to facilitate your understanding of the complex function that glutamate signaling serves in pain. It is likely that glutamate signals through multiple groups of mGluRs at the same synapse in the same "pain" state and the dynamic regulation of each mGluR may determine whether pain is increased (e.g., more Group I signaling) or decreased (e.g., more Group II and III signaling) by glutamate. In some anatomical sites, activation of a specific mGluR such as mGluR1 may increase pain (e.g., spinal cord), while in other sites, activation of this same receptor may decrease pain (e.g., PAG). Many of the studies on pain are done with systemic application of agents which work through mGluRs; combining these studies with those using local application is one way to improve our understanding of where each mGluR plays a specific role. Such an approach is logically appealing, but these agents are seldom as clean (in their target effects) as reported and the physiology is not always as simple as logic would make it seem. Because pain is subjective, understating pain in nonhumans depends on behavioral changes (which themselves may be mediated though neurons expressing and responding to mGluRs) or on measurement of the underlying phenomena (EPSPs, IPSPs) thought to underlie the neuronal communication of pain. In the following sections, we will describe the roles of mGluRs at the level of the peripheral nervous system, the spinal cord, the brainstem, and the brain. Finally, evidence from systemic experiments (e.g., conventional animal knockout approaches) will be evaluated.

3.1 Periphery (nociceptor ending and DRG cell body)

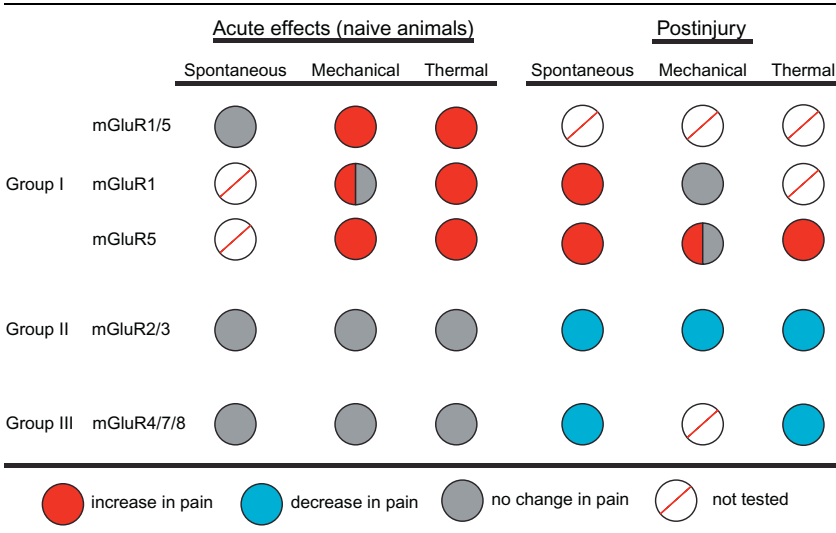
One of the more interesting questions about the role of mGluRs in pain is the extent to which receptors from different families interact and/or are coexpressed on the same cells. In the peripheral nervous system, a coexpression analysis has been only partially completed for a subset of receptors (mGluR1, mGluR2/3, mGluR8).[13] Nonetheless, two important findings have come out of this analysis. First, the number of cells expressing the inhibitory Group II (mGluR2/3; >50% of DRG neurons) and Group III (mGluR8; ~80% of DRG neurons) receptors is considerably higher than cells expressing excitatory Group I (mGluR1; >6%) receptors. Second, there is more overlap (in percent) between Group I-positive cells and either Group II or Group III cells (~50% of mGluR1 cells are double labeled) than between cells expressing Group II and Group III cells (~30% of cells are mGluR2/3 and mGluR8 double labeled). The abundance of inhibitory Group II and III mGluRs on small diameter mGluR1-positive neurons suggests that the push and pull of glutamate within a single nociceptive cell could have a

significant impact on DRG activity and ultimately on pain (Table 2). Keep these data in mind when thinking about the physiological and behavioral consequences of peripheral mGluR signaling described below.

3.1.1 Group I activity in the periphery

Below, we outline evidence that mGluR1 and mGluR5 function in the sensory endings of nociceptors and in the DRG cell bodies themselves. Both mGluR1 and mGluR5 are expressed in unmyelinated and myelinated axons in the periphery of rats and mice[12,14–16] (Fig. 3). Peripheral activation of mGluR1 and mGluR5 with the Group I agonist DHPG (Table 1) causes mechanical allodynia that is blocked with peripheral application of the mGluR1 > mGluR5 antagonist AIDA (Table 1)[16] or the mGluR5

Table 2 Summary of behavioral effects of mGluR activation in the periphery



Specifically, effects are indicated by the color (different gray shades) of the circle. Red (black in the print version) means that activation of the receptor(s) increases pain-like responses, while blue (medium gray in the print version) indicates a reduction in pain-like responses with activation of the indicated receptor(s). A light gray circle represents no change in pain upon activation, and a white circle represents a situation that has not been specifically addressed yet in the literature. In situations with conflicting data (i.e., one study shows an increase in pain and another shows a decrease), circles are half colored (different gray shades). Finally, all circles are representative of the effect of activating the receptor(s), but the actual experimental data may be from a study using antagonists to analyze the function of the receptor. In this table, "postinjury" includes any manipulation to the animal that induces or mimics an injury. Injury can include intraplantar injections of formalin, carrageenan, or capsaicin along with injury models such as chronic constriction injury and spinal cord injury. Spontaneous tests include standard spontaneous assays (e.g., formalin test) as well as newer tests using conditioned place preference unless the conditioning was done with an experimenter-guided mechanical or thermal stimulus.

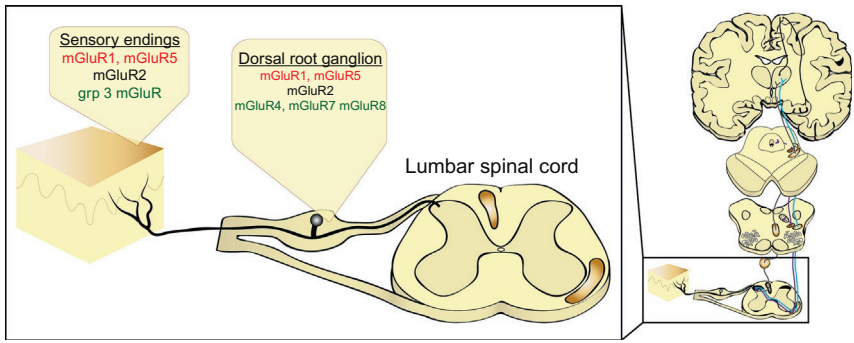


Figure 3 Illustration of mGluR expression in the periphery. mGluRs are expressed in the sensory endings, DRG cell bodies, and/or presynaptic areas in primary sensory neurons. *Image adapted from original art by Maria Elena Morales, used with permission.*

antagonist MPEP but not with the mGluR1 antagonist 4-CPG (Table 1).[15] DHPG also induces thermal hyperalgesia that is blocked with mGluR1 antagonists CPCCOEt/LY367385 (Table 1) or the mGluR5 antagonist MPEP.[12] Intraplantar activation of mGluR5 with the selective agonist CHPG (Table 1) induces mechanical hypersensitivity in naïve animals.[15] In addition, both mGluR1 and mGluR5 at peripheral terminals are involved in spontaneous behavior following intraplantar inflammation.[12,36] Intraplantar blockade of mGluR1 or mGluR5 reduces spontaneous formalin behavior[12]; application of AIDA (mGluR1 > mGluR5 antagonist) decreases spontaneous behavior following intraplantar bee venom application.[36] Interestingly, in the same study, intraplantar AIDA did not reduce or reverse bee venom-induced mechanical hypersensitivity,[36] suggesting that mechanical nocifensive behavior in this pain model is mediated through other receptors, possibly mGluR5. Following intraplantar inflammation with carrageenan, the mGluR5 antagonist MPEP reduces injury-induced mechanical hypersensitivity.[15] In three different models of peripheral nerve injury (total sciatic nerve ligation, partial sciatic nerve ligation, L5 spinal nerve ligation), expression of mGluR5 increases in the injured nerve and DRG neurons.[14] However, mGluR5 inhibition with orally delivered MPEP reversed thermal hypersensitivity in the L5 model only and had no significant effects on mechanical hypersensitivity in any model.[14] As MPEP was delivered systemically, it is unclear whether this effect of MPEP was truly acting in the periphery or whether the effect was central.

In a model of colorectal visceral pain, local peripheral inhibition of mGluR5 can reduce activation of mechanically sensitive nociceptors in

the gastrointestinal tract.[37] In bladder pain, intraperitoneal (IP) inhibition of mGluR5 using MPEP or the negative allosteric modulator (NAM) fenobam (Table 1) decreases nociceptive responses in naïve animals and in animals with a urinary tract infection.[38] This antinociceptive effect may be mediated by central mGluR5 since inhibition of mGluR5 with MPEP directly in the bladder does not alter afferent discharges in response to noxious bladder distention *in vitro*.[39] The peripheral effects of mGluR5, studied via cultured DRG neurons, are mediated, in part, by sensitization of transient receptor potential vanilloid 1 (TRPV1).[8] Interestingly, these effects are mediated through a complex series of intracellular cascades and multiple receptors. This pathway is dependent on PLC, DAG, and PKA but not on PKC.[8] Although there are some disagreements in the data, overall activation of mGluR1 and mGluR5 tends to increase pain-like behavior, and inhibition of these Group I mGluRs can reverse some postinjury pain-like changes (Table 2). Studies using peripherally restricted knockout approaches for mGluR1 or 5 would help fine-tune our knowledge of Group I mGluRs in the periphery.

3.1.2 Group II activity in the periphery

Using a nonspecific antibody for mGluR2/3, there is evidence for expression of mGluR2 and/or mGluR3 in DRG soma and presynaptically in the dorsal horn of the spinal cord[26,28,27] (Fig. 3). mRNA for mGluR3 has not been found in DRG, suggesting that earlier immunostaining was due to mGluR2.[40] A large proportion of DRG neurons that express mGluR2/3 binds the isolectin B4 (IB4)[26] and coexpress TRPV1.[41] Subcutaneous injections of Group II agonists do not alter baseline mechanical[15,42] or thermal sensitivity.[43] In the context of prostaglandin E2- or carrageenan-induced peripheral inflammation, however, the Group II agonist APDC (Table 1) reverses mechanical[42] and thermal hyperalgesia.[43] Intraplantar delivery of the Group II agonist APDC is able to reduce bee-venom-induced spontaneous behavior and mechanical hypersensitivity,[36] and APDC is able to reduce subcutaneous IL-1beta-induced mechanical allodynia.[44] Furthermore, capsaicin-induced spontaneous behaviors are attenuated with intraplantar APDC.[41] Intraplantar blockade of Group II and III mGluRs increases capsaicin-induced spontaneous behaviors, suggesting that during noxious stimulation, there is ongoing antinociceptive activity of Group II and/or Group III signaling in the periphery.[45] The natural product L-acetylcarnitine (LAC), a dietary supplement, reduces allodynia caused by chronic constriction injury.[29] This analgesic effect of LAC is blocked

by the systemically active mGluR2/3 antagonist LY 341495 (Table 1); LAC acts to increase mRNA expression of mGluR2 but not mGluR3 in the spinal cord[29] and in cultured DRG neurons.[46,47] In the spinal cord, mGluR2 is likely acting presynaptically to reduce excitatory neurotransmission in the dorsal horn.[48,49]

Peripheral mGluR2/3 effects have been shown to be cAMP dependent.[43] In cultured DRG neurons, activation of adenylyl cyclase with forskolin resulted in an increase in TTX-resistant current that was blocked by co-application of mGluR2/3 agonist APDC.[50] These data suggest that mGluR2/3 in the periphery is likely coupled to G_i. Corresponding to behavioral findings, Group II agonists reduce capsaicin-induced TRPV1-dependent excitability in a skin-nerve preparation.[41] Overall, activation of Group II mGluRs in the periphery tends to decrease behavioral (Table 2) and electrophysiological manifestations of pain. Although mGluR2 is likely a more predominant player in peripheral pain, future studies with more selective mGluR2 versus mGluR3 antibodies or pharmacological agents will allow for a more complete and discriminative understanding of Group II mGluRs in the peripheral nervous system.

3.1.3 Group III activity in the periphery

Of the Group III mGluRs, the presence of mGlu4, mGlu7, and mGluR8 has been detected in nociceptive primary afferent neurons (Fig. 3). mGluR4 has been found in DRG soma[35] and at presynaptic sites in the spinal cord.[33] This presynaptic staining is presumed to come from primary afferent neurons and spinal interneurons.[33] Similarly, mGluR7 expression has been confirmed in the DRG[35] and primary afferent presynaptic sites in the spinal cord.[34,51] mGluR8 is widely expressed in DRG soma[13] but has not been detected in the spinal cord (on presynaptic or postsynaptic sites).[18] In DRG, approximately 25% of mGluR8-positive cells were co-labeled for TRPV1.[52] Although the functional behavioral role of Group III mGluRs has been studied extensively at the level of the spinal cord, only recently there have been any reports showing peripheral (e.g., intradermal) manipulation of Group III mGluRs in pain. In naïve animals, activation of Group III mGluRs with L-AP4 (Table 1) does not alter baseline mechanical[15] or thermal sensitivity[52] and inhibition of Group III mGluRs with the antagonist UBP1112 (Table 1) does not induce any spontaneous behaviors.[52] In the context of capsaicin injection, two similar studies demonstrated that intraplantar delivery of L-AP4 reduced capsaicin-induced spontaneous behaviors[45] and thermal hyperalgesia.[52] Antagonism of Group II/III or just

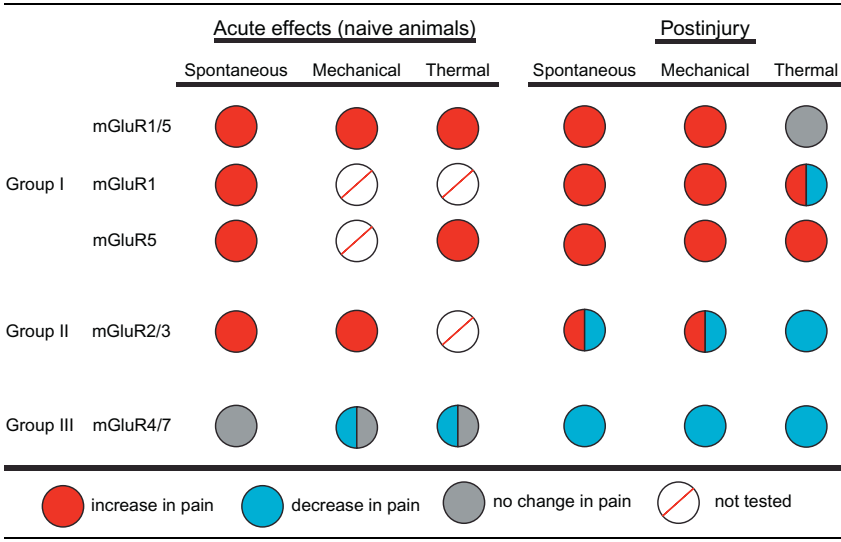
Group III mGluRs enhanced capsaicin-induced pain-like behaviors, and this enhancement was reversed with L-AP4.[45,52] The Group III agonist L-AP4 can reduce forskolin-induced thermal hyperalgesia.[52] Thus, the ability of Group III mGluRs to inhibit TRPV1-induced neuronal activation at the sensory terminal may be through a G_i cAMP-dependent mechanism. Group II/III antagonism in the paw causes thermal hypersensitivity when co-applied with the exogenous ligand L-glutamic acid hydrochloride (GLU) even though GLU does not cause thermal hyperalgesia on its own.[45] Intraplantar delivery of the Group III agonist L-AP4 reduced bee-venom-induced spontaneous nocifensive behavior but did not reduce bee-venom-induced mechanical hypersensitivity.[36] Overall, these data suggest that signaling via Group III mGluRs during peripheral nociceptor activation attenuates thermal pain-like responses.

In an intact skin-nerve preparation, skin-applied capsaicin induces an increase in single unit activity in the nerve.[45] This increase is enhanced with Group III inhibition and attenuated with Group III activation. Corresponding studies from *in vitro* DRG neurons demonstrate an increase in capsaicin-induced Ca^{2+} activity with co-application of a Group II/III antagonist but no effect on Ca^{2+} activity with Group II/III antagonist alone.[45] Application of the Group II/III antagonist alone (no capsaicin) does not change single unit activity in the skin-nerve preparation. Overall, these data suggest that Group III mGluRs are recruited after nociceptor activation (Table 2). The possible role such recruitment has in chronic pain remains unexplored.

3.2 Spinal cord (secondary sensory neurons and interneurons)

The role of mGluRs in nociceptive processing in the spinal cord has been intensely studied. As the first site of central processing of nociceptive signals, mGluRs in the spinal cord play a critical modulatory role in acute nociception and in the transition from acute to chronic pain (Table 3). Primary afferents use glutamate as the primary excitatory neurotransmitter at the afferent-dorsal horn neuron synapse. There are of course many challenges and caveats to studying mGluRs in the spinal cord. These include the presence of presynaptic receptors (e.g., mGluR4/7) on peripheral neurons and the diversity of cell types in the spinal cord (local interneurons, ascending neurons, glia). In the context of this complexity, a picture has emerged during the last 20 years that firmly cements the importance of various mGluRs in the spinal cord processing of pain. In particular, the dynamic modulation of mGluR signaling

Table 3 Summary of behavioral effects of mGluR activation in the spinal cord



Specifically, effects are indicated by the color (different gray shades) of the circle. Red (black in the print version) means that activation of the receptor(s) increases pain-like responses, while blue (medium gray in the print version) indicates a reduction in pain-like responses with activation of the indicated receptor(s). A light gray circle represents no change in pain upon activation, and a white circle represents a situation that has not been specifically addressed yet in the literature. In situations with conflicting data (i.e., one study shows an increase in pain and another shows a decrease), circles are half colored (different gray shades). Finally, all circles are representative of the effect of activating the receptor(s), but the actual experimental data may be from a study using antagonists to analyze the function of the receptor. In this table, "postinjury" includes any manipulation to the animal that induces or mimics an injury. Injury can include intraplantar injections of formalin, carrageenan, or capsaicin along with injury models such as chronic constriction injury and spinal cord injury. Spontaneous tests include standard spontaneous assays (e.g., formalin test) as well as newer tests using conditioned place preference unless the conditioning was done with an experimenter-guided mechanical or thermal stimulus.

appears to play an important role in the development of central sensitization.[53] In the spinal cord, there is evidence for mRNA expression of mGluR1, 5, 2, 3, 4, and 7 but not 6 or 8[18,25] (Fig. 4).

3.2.1 Group I activity in the spinal cord

mGluR1 and mGluR5 have been found to be expressed in a variety of cell types in the dorsal horn of the spinal cord[18] (Fig. 4). This includes both GABAergic interneurons and ascending neurons and glial cells.[27] mGluR1 expression is concentrated in lamina I and II but diffuse mGluR1 staining is found throughout the dorsal horn.[17] mGluR5 is found perisynaptically or postsynaptically on synapses between unmyelinated afferents and spinal cord neurons in lamina I and II.[27,54,55] Similar to the role of Group I mGluRs in

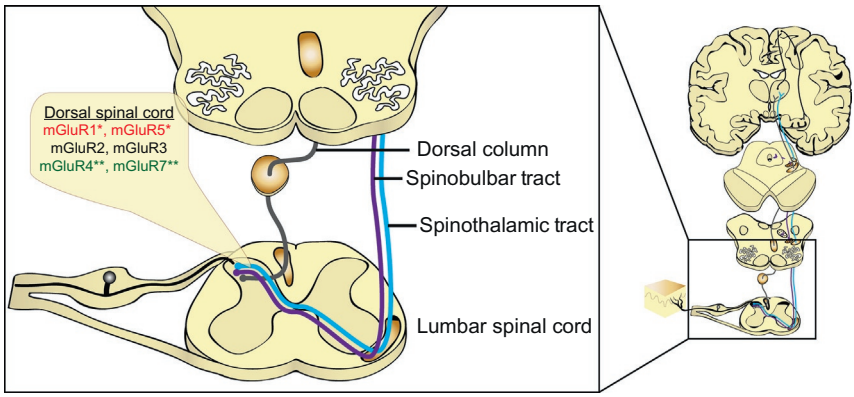


Figure 4 Illustration of mGluR expression in the spinal cord. mGluRs are expressed both presynaptically and/or postsynaptically in the dorsal horn of the spinal cord. *, postsynaptic only (in spinal interneuron or on dorsal horn projection neuron); **, presynaptic only (on primary sensory neuron). *Image adapted from original art by Maria Elena Morales, used with permission.*

the periphery (nociceptors or DRG), activation of mGluR1 and mGluR5 in the dorsal spinal cord tends to increase pain-like behaviors and neuronal excitability. Intrathecal delivery of the Group I agonist DHPG induces mechanical and thermal (hot or cold) hypersensitivity[56,57] as well as spontaneous nocifensive behavior in naïve animals.[58,59] Both mGluR1 and mGluR5 inhibition in the spinal cord can reduce the spontaneous effects of DHPG.[59] Intrathecal DHPG also enhances nocifensive responses to peripheral formalin[60] or complete Freund's adjuvant (CFA) inflammation.[61] Intrathecal inhibition of mGluR5 with MPEP, inhibition of mGluR1 with CPCCOEt, or antisense knockdown of mGluR1 (~40%) reduces pain-like behavior in the formalin test.[59,62]

Suggesting a functional difference in spinal Group I mGluRs after injury, mGluR1 but not mGluR5 expression is increased in the spinal cord following spinal cord injury.[63] Antagonism of mGluR1 but not mGluR5 in the spinal cord reduces mechanical allodynia after this spinal injury, while antagonism of mGluR1 and mGluR5 has opposite effects on thermal hyperalgesia.[64] Inhibition of mGluR1 increases thermal hyperalgesia, while inhibition of mGluR5 decreases thermal hyperalgesia following this spinal injury. However, in other neuropathic models (sciatic nerve injury), mGluR5 expression is increased in lamina II neurons and modulates post-injury thermal hyperalgesia.[14] In another model of nerve injury, chronic constriction injury, intrathecal inhibition of both mGluR1 (with AIDA)

and mGluR5 (with MPEP) reduces mechanical hyperalgesia, and inhibition of mGluR1 reduces cold hyperalgesia.[65] These data from injury models suggest two possibilities. First, distinct injuries may recruit different receptors as part of the long-term changes that accompany the injury. This interpretation would suggest that the efficacy of specific Group I analgesic agents for use in humans would depend in part on the type of pain condition or injury. Alternatively, the differences in expression and function between mGluR1 and mGluR5 after injury may be due to more subtle experimental differences in the studies described above. In this context, caution must be used in interpreting whether one Group I mGluR dominates over the other in chronic pain states.

As postsynaptic modulators, activation of Group I mGluRs tends to depolarize dorsal horn neurons.[10] Both mGluR1 and mGluR5 in the spinal cord are capable of phosphorylating extracellular-signal-regulated kinases 1 and 2 (ERK1/2), which is necessary for spontaneous nocifensive behaviors after intraplantar formalin[59] or CFA[61] inflammation. Subsequent studies identified the potassium leak channel $Kv_{4.2}$ as a critical target downstream of mGluR1/5 and ERK1/2.[10,66] Activation of mGluR1/5 in dorsal horn neurons leads to activation of ERK1/2, which then phosphorylates (and closes) $Kv_{4.2}$. In $Kv_{4.2}$ knockout mice, DHPG does not increase excitability of spinal cord neurons *in vitro* and induces only mild spontaneous nocifensive behavior compared to wild-type mice.[10] Interestingly, the connection between Group I mGluRs and $Kv_{4.2}$ seems to be mediated by mGluR5 only as the mGluR1 antagonist LY367385 does not change DHPG-induced ERK1/2 activation or $Kv_{4.2}$ A-type currents. In this circumstance, the phosphorylation of ERK1/2 by mGluR5 could be PKC and/or PKA dependent,[66] although future studies will need to assess this directly. The intracellular signaling cascade associated with mGluR1 in the spinal cord is still unknown, although the behavioral results described above suggest a role in increasing excitability in dorsal horn neurons. Supporting this, the Group I agonist DHPG enhances excitability of primate dorsal horn neurons during noxious mechanical stimulation under naïve conditions and following peripheral capsaicin stimulation.[67] This facilitation was blocked by the mGluR1 antagonist CPCCOEt, and the effect was actually reversed (i.e., inhibition rather than facilitation of neurons) at high doses of DHPG. The net result of mGluR1 and mGluR5 modulation is to depolarize the neuron, leading to hyperexcitability and long-term potentiation.[53] However, there is also evidence for spinal Group I mGluR activation leading to long-term depression (LTD),[68] which may explain some studies showing

that high levels of the Group I mGluR agonist, DHPG, inhibit spinal neuronal responses to noxious stimulation.[67] Group I mGluR LTD is driven by IP3 production leading to increased intracellular calcium and activation of protein phosphatase I by calcineurin.[68]

Activation of mGluR1 and mGluR5 in the spinal cord tends to increase all manner of pain-like behavior, but there are some intriguing differences in the receptors based on the type of injury (inflammatory vs. neuropathic) or site of injury (peripheral nerve vs. spinal cord), which suggest that there may be subtle but potentially interesting differences between mGluR1 and mGluR5 in the spinal cord (Table 3).

3.2.2 Group II activity in the spinal cord

Although Group II expression is thought to be mainly presynaptic from primary afferents in the spinal cord, there is evidence using nonspecific mGluR2/3 antibodies of postsynaptic staining in the inner part of lamina II[17,27] and on astrocytes[17] (Fig. 4). Group II expression in the spinal cord is decreased following spinal cord injury.[63] Following intradermal CFA inflammation, mGluR2 and mGluR3 mRNA is increased,[69] and following peripheral UV radiation-induced inflammation, mGluR3 mRNA is increased.[25] In the UV radiation study, no change in mRNA for mGluR1, 2, 4, 5, and 7 was found. Intrathecal activation of Group II mGluRs with ACPD (Table 1) induces spontaneous nociceptive behaviors[58] and facilitates formalin pain behavior,[60] but these data are difficult to interpret because of the poor specificity of ACPD for mGluR2/3 (Table 1). More recent data with a selective Group II mGluR agonist, DCG-IV (Table 1), show that mGluR2/3 activation in the spinal cord induces hyperalgesia in paw-pressure nociception in naïve rats.[70] Selective mGluR2/3 inhibition dose-dependently reduces CFA-induced mechanical but not thermal hyperalgesia.[69] In contrast to the pronociceptive data for mGluR2/3 in naïve animals and after peripheral inflammation, mGluR2/3 seems to be antinociceptive in neuropathic models. Following spinal nerve ligation (Chung method), intrathecal DCG-IV decreases injury-induced mechanical hypersensitivity and injury-induced paw-pressure nociception.[70] Pretreatment with the selective Group II agonist APDC (not ACPD) reduced chronic constriction injury-induced mechanical and cold hyperalgesia.[65] Finally, intrathecal APDC can dose-dependently decrease spontaneous nociceptive behaviors driven by intrathecal delivery of the mGluR1/5 agonist DHPG.[71] Overall, activation of spinal Group II mGluRs reduces pain-like responses after neuropathic injury but can, in other circumstances,

increase pain-like responses in naïve or inflammation-injured animals (Table 3). This apparent paradox may be explained by distinct populations of Group II mGluRs in the spinal cord (e.g., pre- vs. postsynaptic).

At the physiological level, Group II receptor agonists depress dorsal horn excitability via a presynaptic mechanism.[49] The Group II antagonist EGLU (Table 1) had a greater inhibitory effect on polysynaptic EPSCs in spinal nerve-ligated animals compared to control animals.[70] Intrathecal Group II blockade suppressed monosynaptic postsynaptic potentials via a presynaptic mechanism.[49] A role of Group II mGluRs in the spinal cord seems to be in reducing GABAergic interneuron activity during increased peripheral nociceptive input.[72] This reduction in GABAergic tone would serve to disinhibit projection neurons and may be a component of the pronociceptive role that Group II mGluRs play in mechanical pressure pain described above.[70]

3.2.3 Group III activity in the spinal cord

There is evidence for expression of mGluR4 and mGluR7 in the spinal cord although both receptors appear to be primarily expressed on the afferent terminals of primary afferent neurons in the superficial laminae of the spinal cord[33,34] (Fig. 4). On a functional level, intrathecal injection of the Group III agonist LAP4 does not induce any spontaneous nociceptive behaviors[58] and does not alter baseline thermal or mechanical sensitivity.[73] Inhibition of spinal Group III mGluRs with MAP4, however, induces mechanical and thermal hyperalgesia in naïve animals.[73] These differential effects of activating Group III mGluRs (no effect) versus inhibiting Group III mGluRs (pronociceptive) in naïve animals may be due to cell-type-specific (glutamatergic vs. GABAergic cells) activity of Group III mGluRs. Specifically, pharmacological activation of Group III mGluRs may produce a net-zero effect by simultaneously reducing both primary sensory neuron excitatory and interneuron inhibitory inputs on dorsal horn neurons. In contrast, inhibition of Group III mGluRs may specifically facilitate glutamate release at the central synapse by reducing ongoing Group III autoinhibition of primary sensory neurons, which would increase pain facilitation. For this hypothesis to be true, it would suggest that presynaptic Group III mGluRs on interneurons are not occupied when the primary sensory neuron is largely quiet (i.e., glutamate release is low).

In fact, Group III mGluR activation has an effect in the context of injury when primary sensory neurons are excited. Pretreatment of the spinal cord with the Group III agonist LAP4 reduces chronic constriction

injury-induced mechanical and cold hyperalgesia[65] and L5/L6 spinal nerve ligation-induced mechanical allodynia.[73] Again, the difference in LAP4 between naïve and injured animals may be due to the increase in primary sensory neuron glutamatergic drive and decrease in spinal cord interneuron inhibitory drive that occurs after neuropathic injury.[74,75] Thus, after injury, LAP4 is able to induce antinociception by reducing excitatory drive on ascending dorsal horn neurons.

Activation of Group III mGluRs by LAP4 in the spinal cord reduces evoked activity to mechanical stimulation (brush, press, pinch) in control conditions and following capsaicin in anesthetized macaques,[76] but LAP4 only reduces evoked activity after injury in rats.[73] These effects after injury were likely mediated by Group III mGluRs in the spinal cord reducing glutamatergic drive on dorsal horn neurons. Activation of the Group III mGluRs with LAP4 suppresses both monosynaptic and polysynaptic EPSPs in spinal cord slices.[49] As mentioned above, a role of Group III mGluRs in the spinal cord seems to be in reducing GABAergic tone during increased peripheral nociceptive input.[49,72] Capsaicin applied to primary nerve endings in a spinal cord preparation inhibits IPSCs. This effect of capsaicin can be blocked by treatment with CPPG, a Group III mGluR antagonist (Table 1).[72] Overall, activation of Group III mGluRs in the spinal cord inhibits nociceptors and interneurons. The determinative factors as to whether activation of Group III mGluRs fails to alter pain-like responses or decrease pain-like responses (Table 3) likely include the state of the primary sensory nociceptor (normal or sensitized), state of the GABAergic interneurons (normal or desensitized), and the expression and localization of mGluRs in these cells. One of the biggest open questions is whether the expression or subcellular localization of these receptors changes on either primary afferents or interneurons. Dynamic changes following injury may explain the complex and confusing activity of Group III mGluRs in the spinal cord.

3.3 Brainstem

As mentioned above, a tremendous amount of research has focused on the processing and modulation of nociceptive information at anatomic loci in the brainstem (e.g., rostral ventral medulla (RVM), PAG, parabrachial nucleus (PB)) and brain (e.g., thalamus, anterior cingulate cortex (ACC), PFC, and amygdala). Not surprisingly, mGluRs have been identified at the expression and functional level in nearly all higher centers in the pain neuraxis[77,78] (Fig. 2). In the following sections, we will review these data

separately for the brainstem (e.g., PAG; Fig. 5) and nociceptive brain areas (Section 3.4; e.g., thalamus, ACC, and amygdala; Fig. 6).

The ascending projections of dorsal horn neurons project primarily to the PAG/PB (via spinobulbar projections) and thalamus (via spinothalamic

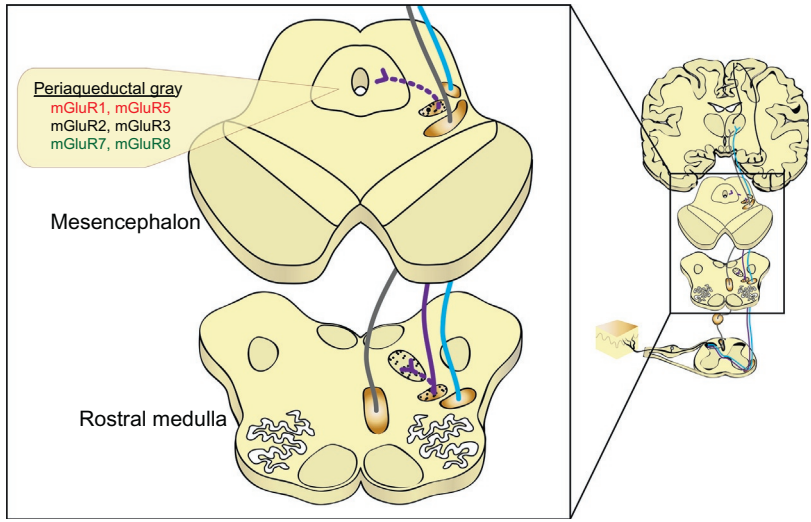


Figure 5 Illustration of mGluR expression in the PAG. mGluRs are expressed in the PAG as part of both the ascending and descending components of the pain neuraxis. *Image adapted from original art by Maria Elena Morales, used with permission.*

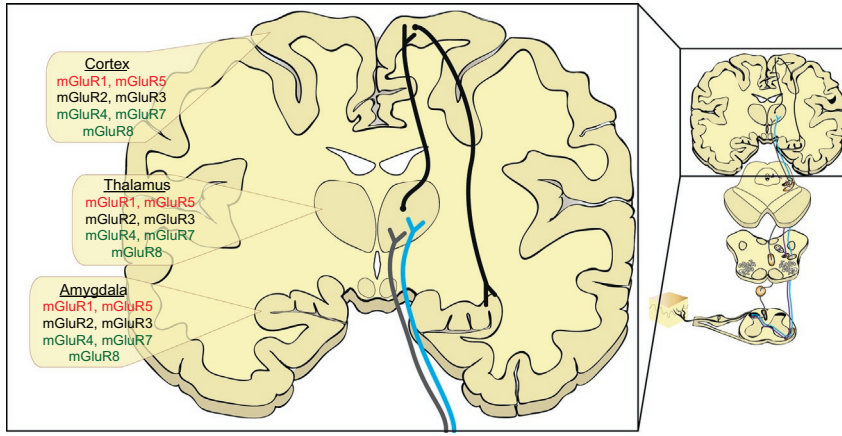
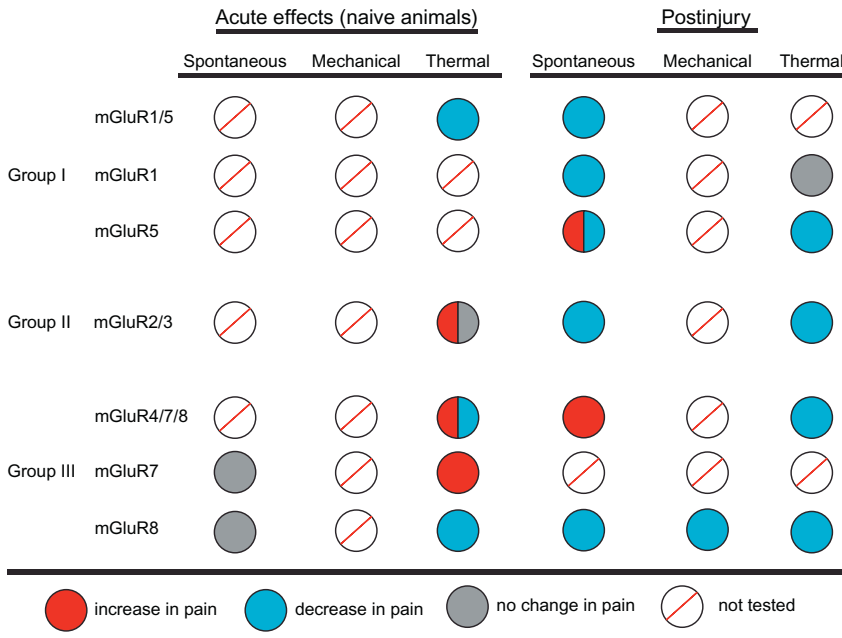


Figure 6 Illustration of mGluR expression in the brain. mGluRs expressed in the thalamus, cortex, and amygdala modulate ascending and descending components of nociceptive information. *Image adapted from original art by Maria Elena Morales, used with permission.*

tract). Modulation at these levels can influence nociceptive input as well as descending output to modulate pain. The behavioral roles of mGluRs in the PAG can be opposite of those found in the periphery, spinal cord, or other areas. For example, the activation of Group I mGluRs (which are pronociceptive in the periphery) causes antinociception, and activation of Group II and III mGluRs (which are antinociceptive in the periphery) causes increased nociception in the PAG (Table 4).

Table 4 Summary of behavioral effects of mGluR activation in the brainstem



Specifically, effects are indicated by the color (different gray shades) of the circle. Red (black in the print version) means that activation of the receptor(s) increases pain-like responses, while blue (medium gray in the print version) indicates a reduction in pain-like responses with activation of the indicated receptor(s). A light gray circle represents no change in pain upon activation, and a white circle represents a situation that has not been specifically addressed yet in the literature. In situations with conflicting data (i.e., one study shows an increase in pain and another shows a decrease), circles are half colored (different gray shades). Finally, all circles are representative of the effect of activating the receptor(s), but the actual experimental data may be from a study using antagonists to analyze the function of the receptor. In this table, "postinjury" includes any manipulation to the animal that induces or mimics an injury. Injury can include intraplantar injections of formalin, carrageenan, or capsaicin along with injury models such as chronic constriction injury and spinal cord injury. Spontaneous tests include standard spontaneous assays (e.g., formalin test) as well as newer tests using conditioned place preference unless the conditioning was done with an experimenter-guided mechanical or thermal stimulus.

3.3.1 Group I activity in the brainstem

At the level of the PAG, mGluR5 receptors seem to play a more prominent role in modulating pain compared to mGluR1 (Table 4). mGluR5 is expressed on dendrites and cell bodies of PAG neurons[19] (Fig. 5). The mGluR1/5 agonist DHPG in the PAG induces antinociceptive behavior in the hot plate test.[78] Low doses of the mGluR5 antagonist MPEP in the dorsal PAG increase nociceptive behavior in the first phase of the formalin test, while higher doses of MPEP decrease nociceptive behavior in the second phase of the formalin test.[79] The antinociceptive effect of high dose MPEP may be related to nonspecific effects of MPEP because DHPG (mGluR1/5 agonist) also decreases nociceptive behavior in the second phase of the formalin test.[80] In addition to mGluR5, the antinociceptive effects of DHPG in the formalin test are also driven by mGluR1 activity.[80]

PAG-directed MPEP but not the mGluR1 antagonist CPCCOEt blocked the antinociceptive effects of cannabinoid agonists in the thermal Hargreaves test.[81] Further suggesting an overall antinociceptive role of mGluR5 in the PAG, the mGluR5 agonist CHPG reduced thermal sensitivity in the Hargreaves assay.[81] One proposed mechanism of mGluR5-induced analgesia in the PAG is via presynaptic inhibition of GABAergic cells. In this scenario, activity-dependent increases in nearby glutamate release "spill over" to GABAergic neurons where presynaptic mGluR5 (on the GABAergic cells) causes circuit disinhibition. In other words, after mGluR5 activation, the subsequent reduction in GABA release disinhibits descending analgesic neurons. There is also evidence from PAG slice recordings for a postsynaptic excitatory role for mGluR1 and mGluR5.[82] Inhibition of mGluR5, but not mGluR1, in the PAG is able to block analgesia induced by PAG delivery of capsaicin.[83] In this scenario, activation of TRPV1 increases glutamate release, which activates mGluR5. mGluR activation leads to PLC-beta activation and yields DAG, which is then converted to the endocannabinoid 2-arachidonoylglycerol (2-AG). 2-AG then activates CB1 receptors to presynaptically reduce GABA release onto PAG analgesic projection neurons. A similar role of mGluR5 in the analgesic effects of intra-PAG neurotensin has also been postulated,[84] suggesting that multiple types of inputs in the PAG may all use mGluR5 as an intermediate in PAG-induced antinociception. This retrograde inhibition of neurons is similar to endocannabinoid and mGluR5-mediated LTD in the hippocampus.[85]

3.3.2 Group II activity in the brainstem

Depending on the pain assay used, there is evidence for both a pronociceptive and an antinociceptive action of Group II mGluRs in the PAG (Table 4). Delivery of the Group II agonist L-CCG-I (Table 1) to the PAG increased pain-like responses in the hot plate test[78] and in the Hargreaves plantar test.[81] In contrast to this pronociceptive role of Group II mGluRs in the PAG, CCG-I reduced spontaneous behavior in formalin test[80] and inhibition of Group II mGluRs with EGLU was able to block the thermal analgesic effects of a cannabinoid agonist.[81] When given alone, EGLU does not alter baseline thermal sensitivity.[81] Although Group II receptors are thought to act presynaptically in the PAG, there is evidence that a small number of PAG neurons respond to postsynaptic Group II mGluR activation.[82] Presynaptic activation of Group II mGluRs with the agonist, DCG-IV, reduced GABA-evoked IPSCs.[86] This regional diversity of Group II mGluRs may explain somewhat contradictory behavioral results during PAG Group II mGluR activation.

3.3.3 Group III activity in the brainstem

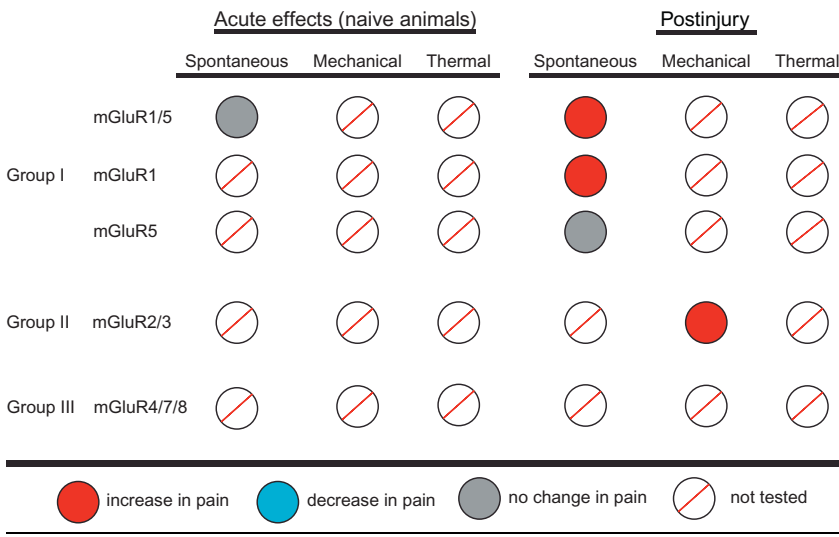
Similar to the dual nature of Group II mGluRs in the brainstem, activation of the Group III mGluRs in the PAG can be pro- or antinociceptive[80] (Table 4). Activation of Group III mGluRs with the agonist L-SOP (Table 1) induces thermal hypersensitivity[78] and increases pain behavior in the second phase of the formalin test.[80] Inhibition of Group III mGluRs with the antagonist MSOP (Table 1) reduced pain-like responses in the second phase of the formalin test.[79] However, MSOP reversed the thermal analgesic effects of a cannabinoid agonist and induced thermal hyperalgesia when used alone,[81] suggesting that Group III mGluRs can also be antinociceptive. In fact, further analyses of Group III mGluRs using receptor-specific agents have revealed some interesting subtleties for the role of these receptors at the level of the PAG. Intra-PAG delivery of the mGluR7 agonist, AMN082 (Table 1), increased pain-like behavior in the hot plate and Hargreaves plantar test.[87,88] Activation of mGluR8 with DCPG (Table 1) decreased pain-like behavior in the hot plate,[88] reduced spontaneous formalin behavior, temporarily blocked carrageenan-induced thermal and mechanical hyperalgesia, and reduced mechanical and thermal hyperalgesia 3 days after chronic constriction injury.[87] Corresponding to the behavioral effects of mGluR7 and mGluR8 in the PAG, activation of these receptors had opposing effects on glutamate release in the PAG and contrasting effects on the RVM ON and RVM OFF cells. Molecularly, activation

of mGluR7 in the PAG causes a decrease in glutamate with a corresponding activation of RVM ON cells and inhibition of RVM OFF cells.[88] mGluR8, by contrast, in the PAG causes an increase in glutamate[89] with an opposite effect on RVM ON and OFF cells.[88] Presynaptic activation of all Group III mGluRs with the agonist, L-AP4, reduced GABA-evoked IPSCs.[86]

3.4 Brain

Due to the complex and subjective nature of chronic pain along with the high incident of affective comorbidities in chronic pain patients,[90] many studies have evaluated the role of mGluRs in modulating the sensory, cognitive, and emotional components of pain in higher brain areas (Fig. 6) such as the thalamus (Table 5), amygdala (Table 6), and PFC and ACC (Table 7).

Table 5 Summary of behavioral effects of mGluR activation in the thalamus



Specifically, effects are indicated by the color (different gray shades) of the circle. Red (black in the print version) means that activation of the receptor(s) increases pain-like responses, while blue (medium gray in the print version) indicates a reduction in pain-like responses with activation of the indicated receptor(s). A light gray circle represents no change in pain upon activation, and a white circle represents a situation that has not been specifically addressed yet in the literature. In situations with conflicting data (i.e., one study shows an increase in pain and another shows a decrease), circles are half colored (different gray shades). Finally, all circles are representative of the effect of activating the receptor(s), but the actual experimental data may be from a study using antagonists to analyze the function of the receptor. In this table, "postinjury" includes any manipulation to the animal that induces or mimics an injury. Injury can include intraplantar injections of formalin, carrageenan, or capsaicin along with injury models such as chronic constriction injury and spinal cord injury. Spontaneous tests include standard spontaneous assays (e.g., formalin test) as well as newer tests using conditioned place preference unless the conditioning was done with an experimenter-guided mechanical or thermal stimulus.

Table 6 Summary of behavioral effects of mGluR activation in the amygdala

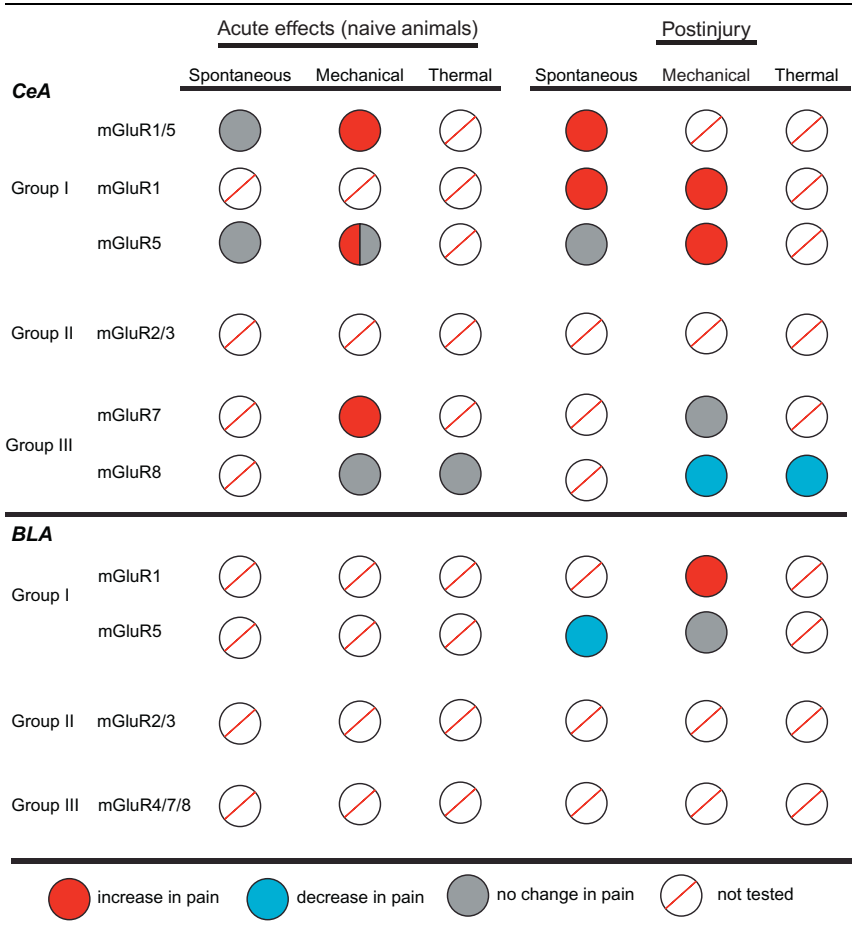


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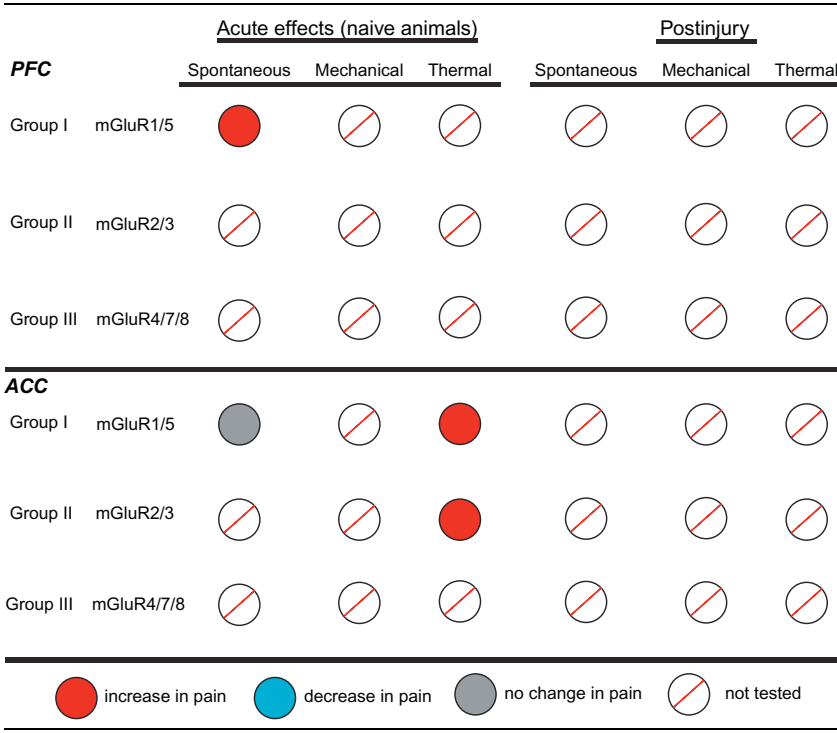
Table 7 Summary of behavioral effects of mGluR activation in the cortex



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3.4.1 Group I activity in cortical and subcortical areas

In the thalamus, Group I mGluRs are widely expressed with prominent expression of mGluR1 in the ventroposterolateral (VPL) and ventroposteromedial (VPM) nuclei[20] (Fig. 6). Chronic constriction injury induces increases in expression of both mGluR1 and mGluR5 in the thalamus.[11] Administration of the Group I agonist DHPG in the VPL enhanced pain

responses in both the first and second phases of the formalin test; this DHPG enhancement of the second phase of the test was dependent on PLC-beta.[91] However, DHPG alone in the VPL (i.e., no formalin) did not induce any spontaneous pain-like behaviors. Furthermore, VPL administration of mGluR1 antagonists (CPCCOEt or AIDA) but not an mGluR5 antagonist (MPEP) reduced pain-like behavior in the second phase of the formalin test. Consistent with a local role in the thalamus for mGluR1 but not mGluR5, it was shown that systemic (IV) but not local thalamic administration of the mGluR5 antagonist MPEP reduced mechanical noxious stimulation-induced excitability of VPL neurons.[92] However, another study found that thermal-induced excitability of thalamic neurons was attenuated by local thalamic administration of both mGluR1 and mGluR5 antagonists.[93]

In the last decade, the amygdala has been a site of intense study of Group I mGluRs in the modulation of pain (Table 6). In particular, distinct roles of Group I mGluRs in the central nucleus of the amygdala (CeA) and basolateral nucleus of the amygdala (BLA) have been described (Fig. 6). The CeA receives direct nociceptive information from the spinal cord and indirect signals from the thalamus and PB.[94] mGluR1 and mGluR5 expression is increased in the CeA following peripheral injury.[21] Activation of mGluR1/5 with DHPG is sufficient to increase somatic and visceral pain-like responses in naïve animals[95–97] via mGluR5 receptors but induces aversion in a pain-related conditioned aversion task acting primarily through mGluR1 receptors.[98] Inhibition of mGluR5 does not alter spontaneous formalin behavior or somatic sensitivity in naïve animals,[96] but the mGluR5 antagonist, MPEP, does decrease responses to acute noxious visceral bladder distention.[95] In the context of spared nerve injury, the mGluR1/5 agonist DHPG facilitated and the mGluR1 antagonist CPCCOEt inhibited pain-related conditioned aversion.[98] Inhibition of mGluR5 through amygdala-specific conditional deletion of mGluR5 did not alter spontaneous formalin behavior but did decrease formalin-induced mechanical hypersensitivity.[96] In an arthritis model of pain, mGluR1 or mGluR5 antagonists had inhibitory effects on ultrasonic or audible vocalizations, respectively, to noxious mechanical stimulation, while mGluR1 but not mGluR5 inhibition reversed arthritis-induced mechanical hypersensitivity.[99] There is evidence that the role of mGluR1/5 in the modulation of pain may be lateralized to the right amygdala. Specifically, inhibition of mGluR5 in the right but not left amygdala with MPEP decreased formalin-induced mechanical hypersensitivity.[96] These data are consistent with reports of lateralization of ERK1/2 activation in the amygdala during injury[100] and

physiological data showing that right amygdala neurons have larger nociceptive receptive fields before and after injury compared to left amygdala neurons.[101] Using a mixture of pharmacological and electrophysiological data, the molecular signaling pathway of Group I mGluRs in the amygdala has emerged.

It appears that mGluR1 signals primarily presynaptically (in GABAergic cells) and mGluR5 signals postsynaptically (in CeA neurons).[21] A group of neurons located in the lateral capsular division of the CeA (CeLC) respond to noxious stimuli and show an increase of excitatory transmission relative to inhibitory transmission following arthritis induction.[102] Under acute noxious (i.e., uninjured) circumstances, only mGluR5 antagonists can block nociceptive responses in the CeLC.[103] After injury, by contrast, both mGluR5 and mGluR1 antagonists can reduce the injury-induced hyperexcitability.[21,103] mGluR1 appears to act presynaptically to inhibit GABAergic neurons whose cell bodies lie in the intercalated cell mass outside the CeA.[102] This activity disinhibits neurons within the CeLC. On the other hand, mGluR5 increases excitability postsynaptically on CeA neurons.[102] mGluR5 effects are dependent on IP3 (but not PKC) leading to reactive oxygen species (ROS) production and activation of ERK1/2 and PKA.[96,97] mGluR1 action in the CeA is dependent on Homer1a[104] signaling and ROS production.[105]

In the BLA, inhibition of mGluR1 (with CPCCOEt) but not mGluR5 (with MPEP) reduces mechanical allodynia associated with an arthritis model.[106] The role of Group I mGluRs in the BLA is related to role of these receptors in the PFC and plays a significant role in the modulation of decision making during pain.[107] After painful injury, medial PFC neurons are inhibited and this is thought to disrupt normal decision making. Activation of mGluR1/5 with DHPG in the BLA depresses PFC neurons through a BLA mGluR1-dependent mechanism.[106] Activation with DHPG in the PFC but not the ACC can impair decision making as is seen in chronic pain states.[22] Chronic pain impairs decision making through a physiological inhibition of PFC pyramidal neurons.[107] The physiological inhibition can be mimicked in naïve animals using the mGluR1/5 agonist DHPG directed at the PFC and can be reversed with an mGluR1 antagonist (LY367385) but not an mGluR5 antagonist (MPEP) in the PFC.[108] Chronic constriction injury induces increases in expression of mGluR1 along with Homer scaffold proteins in the PFC.[11]

Activation of Group I and Group II mGluRs in the ACC with ACPD facilitates pain-like behavior in the tail-flick reflex.[109] Following painful

peripheral amputation, LTD in the ACC is impaired.[110] This impairment of LTD is that the ACC is coupled with findings showing an enhancement of excitatory synaptic activity in the ACC after injury. Interestingly, the loss of LTD is related to signaling via mGluR1. Although activation of mGluR1 on its own cannot induce LTD in amputated animals, mGluR1 activation can prime the system for LFS (low-frequency stimulation)-induced LTD. DHPG can prime LFS–LTD after injury, and this effect is blocked with the mGluR1 antagonist, LY367385, but not an mGluR5 antagonist.[110]

3.4.2 Group II activity in cortical and subcortical areas

Moderate Group II mGluR staining is found in presynaptic and postsynaptic sites throughout the brain.[111] Although lack of specific antibodies has limited the specific characterization of mGluR2 versus mGluR3, evidence from mGluR2 knockout animals (i.e., mGluR3 staining only) has shown that both receptors are expressed at high levels throughout brain areas involved in pain processing (Fig. 6). Group II mGluRs are expressed at low levels in the nociceptive components of the thalamus[20,28] including the reticular thalamus. Inhibition of Group II mGluRs in the reticular thalamus with the antagonist, EGLU, reduces mechanical allodynia in an arthritic model.[112] No other studies to date have probed the behavioral (Table 5), physiological, or molecular implications of Group II mGluRs in the thalamus leaving this as an important area for future study.[20] Nonetheless, the available data suggest that Group II mGluRs in the thalamus serve a pronociceptive role during injury. It has been hypothesized that these receptors inhibit GABAergic cells in the thalamus.[112,113] Thus, inhibition of Group II mGluRs with EGLU would serve to disinhibit GABAergic cells causing a reduction in the excitability of thalamic projection neurons and a reduction in allodynia.

In the CeA, the Group II agonist LY354740 reduces EPSCs in normal animals via a presynaptic mechanism; this effect is enhanced in slices from animals with arthritis.[114] Extracellular recordings from the CeA in injured animals show that Group II activation can reduce responses to noxious stimulation of the knee in arthritis.[115] The effects of the agonist are enhanced in injured animals relative to normal animals but only for noxious stimulation. Inhibitory effects of Group II activation during innocuous stimulation do not differ based on the pain state. As Group II mGluRs are classically thought to be G_i coupled, modulation of PKA via Group II mGluR signaling in the CeA may counterbalance mGluR1 signaling, which is PKA dependent in the CeA.[97]

Following arthritis-like injury, mGluR3 mRNA is increased throughout the cortex with noticeably strong increases in the ACC.[116] As mentioned above, the dietary supplement LAC decreases mechanical allodynia through a mechanism involving mGluR2 receptors. LAC treatment also selectively increases expression of Group II protein in the cerebral cortex.[117] This may be related to emotion and cognition during pain. Increased expression of Group II mGluRs with LAC could serve to inhibit excitatory cortical areas and would thereby lessen the impact of noxious stimuli on cognition and emotion. Otherwise, no other behavioral (Table 7) or electrophysiological studies have evaluated Group II mGluRs in the ACC or other cortical areas in the context of pain.

3.4.3 Group III activity in cortical and subcortical areas

mGluR4, mGluR7, and mGluR8 are expressed in the thalamus, amygdala, frontal cortex, and ACC[35] (Fig. 6). Similar to the Group II mGluRs, Group III mGluRs are expressed at low levels in the nociceptive components of the thalamus.[20,118] mGluR4 and mGluR7 predominate in the VPL/VPM with mGluR7 and mGluR8 also expressed in the reticular thalamic nucleus.[20] No study to date has systematically studied the behavioral (Table 5), physiological, or molecular implications of Group III mGluRs in the thalamus.

mGluR8 expression is increased in the CeA following arthritis injury in animals.[119] mGluR7 and mGluR8 in the CeA have differential effects on pain-like behavior depending on the pain state of the animal (Table 6). In naïve animals, mGluR7 (AMN082) but not mGluR8 (DCPG) agonists in the CeA increase pain-like responses to noxious mechanical stimulation.[119,120] After arthritis injury, by contrast, mGluR8 but not mGluR7 activation actually decreases pain-like responses to mechanical stimulation[120] and mGluR8 activation decreases responses to thermal stimulation after intraplantar carrageenan.[119] These opposing roles for mGluR7 and mGluR8 in pain are similar to the effects of mGluR7 versus mGluR8 in the PAG (see above Section 3.3.3). Somewhat surprisingly, this antinociceptive effect of pharmacological activation of mGluR8 in the CeA is coupled to an increase in CeA glutamate and decrease in GABA.[119] This association is surprising because a similar effect on neurotransmitters has been postulated for the opposite behavioral effects of Group I mGluR activation in the CeA. In extracellular recordings from the CeA in injured animals, Group III activation reduces responses to noxious stimulation of the knee in arthritis,[115] but unlike Group II mGluRs, activation of Group III mGluRs causes greater inhibition of CeA neurons to innocuous stimulation

after injury. In slices from arthritic animals, mGluR8 activation reduces evoked EPSCs, while mGluR7 activation with AMN082 increases glutamatergic signaling by presynaptic inhibition of GABAergic neurons.[121] It should be noted that the evolving role of Group III mGluRs in the CeA is missing important data on other members of this mGluR family in the amygdala. Group III mRNA is increased in arthritic rats in the ACC, but no studies to date have analyzed these receptors in pain-like behavior (Table 7) or physiology.[116]

3.5 Conventional knockout and systemic effects

A number of scientific studies have evaluated the role of mGluRs in pain at an organismal level. These include studies looking at whole-animal genetic "knockouts" of *mGluR* genes or systemic delivery of pharmacological agents to an animal. (*Note*: All of the mGluRs have been genetically knocked-out out, but only a few have been evaluated for pain-like changes.) The drawback of these systemic studies, of course, is that by treating the entire animal, region-specific information is hard to obtain. Any developmental compensation for the knockout may also give rise to a different phenotype than other functional studies reveal. On the other hand, studies of the systemic effects of blocking or activating different mGluRs can be useful in predicting outcomes in future clinical trials with these or new agents, including revealing important side effects of mGluR activation or inhibition outside of the pain system. In particular, as we have seen, there are some areas of the pain neuraxis where the "typical" roles of the mGluRs seem to reverse. For example, while mGluR1 and mGluR5 are pronociceptive throughout most parts of the body, they seem to be antinociceptive in the PAG. Thus, a systemically delivered antagonist could reveal which one of these roles (i.e., pro- vs. antinociceptive) might predominate during treatment of a pain patient. What follows are a few examples showing that systemic manipulation of mGluRs tends to yield effects in pain that are in line with the most well-described role of a particular receptor. mGluR5 appears *in vivo* to be primarily pronociceptive, and inhibition of mGluR5 systemically tends to decrease pain. This suggests that the antinociceptive role of mGluR5 in the PAG may not be of serious clinical consequence in the context of chronic pain or injury with systemic treatments.

During noxious stimulation of a rat's paw, IV administration of the mGluR5 antagonist MPEP but not the mGluR1 antagonist AIDA reduces excitatory responses in VPL thalamic neurons.[92] Conventional knockouts

(i.e., whole body deletion from conception) for mGluR5 have a complex phenotype that includes learning and memory abnormalities[122] and overall hypermobility.[123] Although a complete analysis of these mice in the context of pain is ongoing, published data show that conventional knockout of mGluR5 leads to a reduction in both the first and second phases of the spontaneous formalin test, a small amount of baseline mechanical hyposensitivity, a partial reduction in formalin-induced mechanical hypersensitivity,[96] and reduced visceromotor responses to noxious bladder distention.[38] This physiological effect was similar to reduction in visceromotor responses with systemic application of the mGluR5 antagonist, fenobam.[38] Recently, we found that systemic delivery of either fenobam- or MPEP-induced conditioned place preference in spared nerve injured male and female mice but not in sham-operated mice.[124] These data suggest that inhibition of mGluR5 may be effective in providing relief from spontaneous negative stimulation after nerve injury. Furthermore, the observation that sham-operated mice did not show preference for fenobam or MPEP suggests that unlike morphine, mGluR5-inhibiting drugs do not provide positive reinforcement in the absence of injury.

Most of the observed phenotype in mGluR2 and mGluR3 conventional knockouts supports a more prominent role of mGluR2 rather than mGluR3 in pain.[29] mGluR2 knockout mice exhibit exaggerated behavior in the second phase of the formalin test, while mGluR3KO mice are not different from wild-type littermates.[29] Furthermore, the established analgesic effects of the nonspecific mGluR2/mGluR3 agonist LY354740 (Table 1) are blocked in mGluR2 knockouts only.[29] Systemic (IP) delivery of three different Group II mGluR agonists reduced spontaneous formalin behavior and mechanical allodynia in nerve-ligated rats.[125]

Systemic activation of mGluR8 reduced the second phase of formalin test and mechanical/thermal hyperalgesia after carrageenan injection.[87] This effect on the formalin test was reversed with intra-PAG delivery of the Group III antagonist, MSOP.

3.6 mGluR drug development

As described above, mGluRs impact nearly every anatomical stop along the pain neuraxis. G_q-coupled Group I mGluRs tend to increase pain (in naïve and injured conditions) and are likely involved in pain chronicity. In contrast, G_i-coupled Group II and III mGluRs are typically analgesic after injury. As such, there is considerable interest in targeting the mGluR system

to treat chronic pain in humans. This quest is furthered by the fact that mGluR signaling is also heavily involved in other conditions such as schizophrenia, depression, anxiety, epilepsy, and fragile-X syndrome. The obvious advantage of targeting mGluRs as opposed to iGluRs (e.g., NMDA receptors) is that mGluRs primarily have a modulatory role; thus, mGluR modulation would be less likely to affect normal functioning. This fact is particularly true for positive allosteric modulators (PAMs) and NAMs, which due to their nonligand-binding site interaction with receptors will only have an effect when glutamate is bound to the receptor (i.e., if the ligand is not there, the drug will have minimal effect). Clinical trials for specific mGluR agents have been limited to schizophrenia and fragile-X syndrome. However, preclinical modeling of mGluR agents for human use in pain is well underway.

3.6.1 Group I mGluR drug development

One of the more promising drugs for testing in human pain patients is the NAM fenobam. This drug was originally developed by McNeil Laboratories in the 1970s as an anxiolytic agent.[126] Taken through phase II clinical trials, it was eventually dropped because of low anxiolytic effects in patients and psychostimulant side effects.[127] Interest in fenobam has been renewed in the last 10 years, however, when it was shown to be a potent antagonist for mGluR5.[126] In animal models, fenobam reduces pain in the formalin test.[128] The specificity of the agent for mGluR5 seems better compared to the prototypical mGluR5 antagonist, MPEP. Specifically, when MPEP or fenobam was given to mGluR5 conventional knockout animals, only MPEP reduced spontaneous formalin behavior below the knockout vehicle control mice.[128] Consistent with its original development, fenobam also reduces anxiety-like behavior in mice,[123] a property that may be advantageous in pain clinical trials. The major side effect of fenobam appears to include hypermobility in a number of locomotor assays. However, data from our lab suggest that mice quickly develop tolerance to the locomotor but not to the analgesic effects of the drug.[124] To date, one modern study has used fenobam in human patients. In a study of fragile-X syndrome (characterized in part by hyperactivity of mGluR5), a single dose of fenobam did not produce any adverse events and improved prepulse inhibition is a subset of patients.[129]

In addition to specific agents for mGluR1 or mGluR5, there is considerable interest in agents that target Group I mGluRs and other receptors at the same time. A recent study described the development of MMG22 (Table 1), which is an mGluR5 antagonist and mu opioid receptor

(MOR) agonist.[130] Such an agent could provide improved benefits to patients by simultaneous reducing mGluR5 and increasing MOR signaling to induce analgesia. One of the more exciting tenets of such a strategy is that the mGluR5 antagonist could reduce the dose of MOR agonist needed, which may reduce unwanted side effects of mu agonists including addiction and tolerance. Of additional interest of this specific agent is that it likely targets mGluR5–MOR heteromers, which have been identified previously in culture models only.[131] In this new study, MMG22 reduced mechanical allodynia in lipopolysaccharide-injected animals.[130]

3.6.2 Group II mGluR drug development

As described above, activation of Group II mGluRs at many points in the pain neuraxis is analgesic. Changes in Group II mGluRs (expression and/or function) are suspected in chronic conditions and in the normal healing process. To date, no clinical trials in pain have been reported for specific Group II mGluR agents. A phase II double-blind study of the mGluR2/3 agonist LY404039 (Table 1) in schizophrenia demonstrated that the compound was well tolerated and had antipsychotic effects compared to placebo in patients.[132,133] The antipsychostimulant effects of Group II mGluR activation is in contrast to the early trials for mGluR5 inhibition in humans, which showed psychostimulant effects in a double-blind placebo-controlled study.[127] Although there are more data supporting the role of Group I mGluRs in pain compared to Group II mGluRs, it is worth considering additional focus on the Group II mGluRs in the treatment of pain in humans. LAC, the dietary supplement, has been reported to reduce pain in humans[134] through an action that may depend on activation of mGluR2.[117] Recent evidence suggests that mGluR2 may heterodimerize with the serotonin receptor 5-HT_{2A}.[135] 5-HT_{2A} has been shown to dynamically alter pain based in part on condition and site of modulation (peripheral vs. central nervous system).[136] As such, the existence of an mGluR2/5-HT_{2A} heterodimer (similar to the story described above for mGluR5 and MOR) may prove to be advantageous for treatment of pain conditions.

3.6.3 Group III mGluR drug development

As the most diverse group of mGluRs, there is considerable interest in developing agents with specificity for different Group III mGluRs for the treatment of pain. Future clinical studies are likely to investigate targeting of Group III mGluRs to reduce pain through activation of the receptors.

4. CONCLUSION

With a distribution that ranges from the nociceptive sensory terminal, DRG, spinal cord, descending pain modulatory centers all the way to higher cognitive processing centers, mGluRs have been shown to be important in the normal and pathological processing of noxious information. The diversity of actions of the mGluRs as they excite or inhibit cells through pre-, post-, or perisynaptic mechanisms on the soma, dendrites, or axons of primary, secondary, and tertiary nociceptive neurons suggests that there are still many undiscovered or poorly understood roles for the mGluRs in the pain neuraxis. In general, Group I mGluRs (mGluR1 and mGluR5) play a pronociceptive role through excitatory modulation at pre- or postsynaptic sites. Group II (mGluR2 and mGluR3) and Group III mGluRs (mGluR4, mGluR6, mGluR7, mGluR8), by contrast, tend to reduce pain through a presynaptic mechanism. Given this distribution, it may be that an ideal drug would be one that acts as an antagonist at Group I mGluRs and an agonist at Group II/III mGluRs. The signaling pathways involved in the modulation of pain by mGluRs have only been studied in the periphery and spinal cord. Even with the paucity of data on the subject, it is clear that a given receptor can act through both canonical and noncanonical signaling cascades to induce an effect. The future of mGluRs in pain will rest on the ability of basic scientists and clinicians to transition from the fragmentary evidence presented above into clinically relevant and meaningful treatment paradigms.

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