WCP2023

2 - 7 JULY 2023

GLASGOW | SCOTLAND

19TH WORLD CONGRESS OF BASIC AND CLINICAL PHARMACOLOGY

Register now

You could save nearly 40%*

*Early bird deadline: 17 March 2023

wcp2023.org

BRITISH PHARMACOLOGICAL SOCIETY

IUPHAR INTERNATIONAL UNION OF BASIC AND CLINICAL PHARMACOLOGY

Visit Scotland | Alba™ Business Events

GLASGOW CONVENTION BUREAU

Received: 27 November 2020 | Revised: 12 March 2021 | Accepted: 31 March 2021

DOI: 10.1111/bph.15514

Themed Issue: cGMP Signalling in Cell Growth and Survival

COMMISSIONED REVIEW ARTICLE - THEMED ISSUE

cGMP signalling in dorsal root ganglia and the spinal cord: Various functions in development and adulthood

Hannes Schmidt[1] | Alexandra Böttcher[1] | Tilman Gross[2] | Achim Schmidtko[2]

[1]Interfaculty Institute of Biochemistry, University of Tübingen, Tübingen, Germany

[2]Institute of Pharmacology and Clinical Pharmacy, Goethe University, Frankfurt am Main, Germany

Correspondence
Hannes Schmidt, Interfaculty Institute of Biochemistry, University of Tübingen, Auf der Morgenstelle 34, D-72076 Tübingen, Germany.
Email: hannes.schmidt@uni-tuebingen.de

Achim Schmidtko, Institute of Pharmacology and Clinical Pharmacy, Goethe University, Max-von-Laue-Str. 9, D-60438, Frankfurt am Main, Germany.
Email: schmidtko@em.uni-frankfurt.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Numbers: SCHM 2629/4-1, SCHM 2629/3-1, 335549539/GRK2381, SCHM 2371/1

Cyclic GMP (cGMP) is a second messenger that regulates numerous physiological and pathophysiological processes. In recent years, more and more studies have uncovered multiple roles of cGMP signalling pathways in the somatosensory system. Accumulating evidence suggests that cGMP regulates different cellular processes from embryonic development through to adulthood. During embryonic development, a cGMP-dependent signalling cascade in the trunk sensory system is essential for axon bifurcation, a specific form of branching of somatosensory axons. In adulthood, various cGMP signalling pathways in distinct cell populations of sensory neurons and dorsal horn neurons in the spinal cord play an important role in the processing of pain and itch. Some of the involved enzymes might serve as a target for future therapies. In this review, we summarise the knowledge regarding cGMP-dependent signalling pathways in dorsal root ganglia and the spinal cord during embryonic development and adulthood, and the potential of targeting these pathways.

LINKED ARTICLES: This article is part of a themed issue on cGMP Signalling in Cell Growth and Survival. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v179.11/issuetoc

KEYWORDS
axon bifurcation, axon branching, cGMP signalling, dorsal root ganglia, itch, pain, somatosensory system, spinal cord

1 | INTRODUCTION

The somatosensory system consists of receptors and neural pathways through which the body detects and processes information about pain (nociception), itch (pruriception), temperature (thermosensation), touch (mechanosensation), head and body position/balance (proprioception) and head and body movement (kinesthesia). A somatosensory circuit is typically composed of first-order, second-order and tertiary-order neurons which relay and process stimuli detected by distinct receptors in the skin, muscles, tendons or internal organs via afferent connections to the spinal cord and hindbrain, and further to brain structures like the cerebellum, thalamus or cortex

Abbreviations: 8-Br-cGMP, 8-bromo-cGMP; BNP, B-type natriuretic peptide, natriuretic peptide B; CFA, complete Freund's adjuvant; CNG, cyclic nucleotide-gated; CNP, C-type natriuretic peptide, natriuretic peptide C; CSG, cranial sensory ganglia; DCC, deleted in colorectal cancer; DREZ,, dorsal root entry zone; DRG, dorsal root ganglia; E, embryonic day; GC-1, guanylyl cyclase $\alpha_1\beta_1$; GC-2, guanylyl cyclase $\alpha_2\beta_1$; GC-A, guanylyl cyclase A; GC-B, guanylyl cyclase B; GRP, gastrin-releasing peptide; HNOX, haem NO/oxygen binding; MTN, mesencephalic trigeminal neurons; NO, nitric oxide; NO-GC, nitric oxide-sensitive guanylyl cyclase; NOS, NO synthase; Npr, natriuretic peptide receptor; PDE, phosphodiesterase; pGC, particulate guanylyl cyclase; PKG, protein kinase G; ROS, reactive oxygen species.

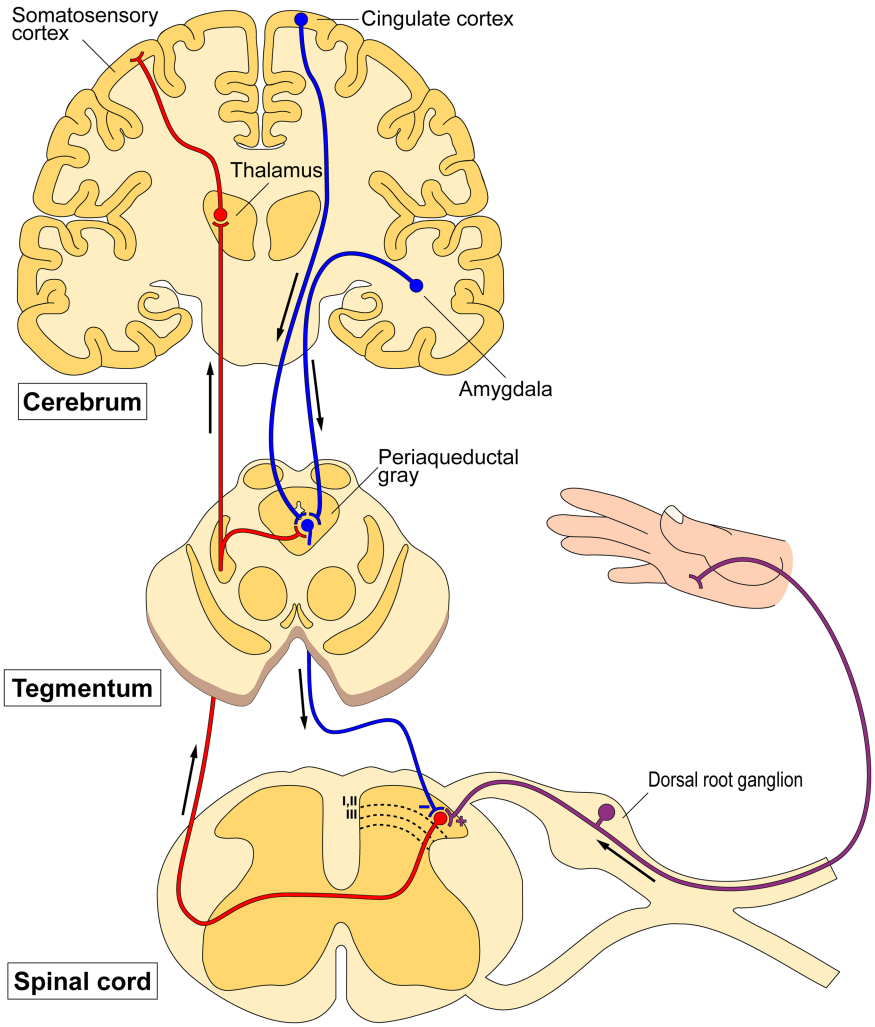

FIGURE 1 Anatomy of the somatosensory system. As an example, a scheme of the nociceptive system is shown. Primary afferent neurons (pink) convey sensory information from the skin and other body regions to the dorsal horn of the spinal cord, where the incoming input is modulated by excitatory and inhibitory interneurons and projection neurons. Many dorsal horn projection neurons (red) have axons that cross the midline and travel rostrally in the contralateral white matter to terminate in the thalamus, the periaqueductal gray matter and other supraspinal sites, and the information is further transmitted to the somatosensory cortex. Although most projections of dorsal horn neurons are to contralateral loci, bilateral and ipsilateral projections have been described (Choi et al., 2020; Spike et al., 2003) (not shown). A network of brain structures including the cingulate cortex and the amygdala contribute to the affective component of somatosensation. In addition, pathways from the periaqueductal grey and other brain structures descend to the dorsal horn (blue) and modulate the output from the spinal cord. For a detailed review of the nociceptive system, see Peirs and Seal (2016) and Todd (2010)

(Figure 1). During embryonic development of the somatosensory system, the primary sensory neurons of the trunk originate from multipotent neural crest progenitor cells that migrate from the dorsal neural tube to the nascent dorsal root ganglia (DRG), whereas the neurons of the cranial sensory ganglia (CSG) are mainly derived from a subset of ectodermal thickenings termed neurogenic placodes, and from the cranial neural crest. The neurons of the DRG and the CSG extend a peripheral branch and a central projection whose collateral branches establish synaptic contacts with second-order neurons in the spinal cord or hindbrain, respectively (Jacobs, 2018).

Accumulating evidence suggests that the second messenger **cGMP** plays an important role during embryonic development of the somatosensory system and for the processing of distinct sensations in the adult. In general, cGMP is generated by two types of enzymes, the **transmembrane (particulate) guanylyl cyclases** (pGCs) and **nitric oxide-sensitive guanylyl cyclases** (also termed soluble guanylyl cyclases, sGCs; we will use the abbreviation 'NO-GC' in this review). Mammals express seven pGC forms (**GC-A**, **GC-B**, **GC-C**, **GC-D**, **GC-E**, **GC-F** and **GC-G**), but GC-D and -G are pseudogenes in humans (Kuhn, 2016; Potter, 2011). Stimulators of pGCs include natriuretic peptides, **guanylin**, **uroguanylin** and **Escherichia coli heat-stable enterotoxin** (Hofmann, 2020). The two known forms of NO-GC are heterodimers composed of either an α_1 and β_1 subunit (**GC-1**) or an α_2 and β_1 subunit (**GC-2**) and are stimulated by nitric oxide (NO) and carbon monoxide (Koesling et al., 2016). Effectors of cGMP include **protein kinase G 1** (PKG1; also termed cGMP-dependent protein kinase I, cGKI), **protein kinase G 2** (PKG2; also termed cGMP-dependent protein kinase II, cGKII), **cyclic nucleotide-gated (CNG) channels** and cGMP-regulated **phosphodiesterases** (PDEs) such as PDE2A, **PDE3A** and PDE3B. The degradation of cGMP is mediated by cGMP-hydrolyzing PDEs, of which some are specific for cGMP, that is, PDE1A, PDE1B, **PDE5A**, PDE6 isoenzymes and PDE9A, whereas PDE1C, PDE2A, PDE3A, PDE3B, **PDE10A** and PDE11A hydrolyse both cGMP and **cAMP** (Hofmann, 2020). Moreover, accumulating evidence indicates that intracellular cGMP levels are also regulated by efflux through ATP-dependent transporters (Aronsen et al., 2014; Cheepala et al., 2013) and that cGMP might act as an extracellular signalling molecule (Cabrera-Pastor et al., 2017; Castro et al., 2013; Jin et al., 2001; Zimmerman et al., 2008). However, as the evidence for extracellular cGMP in DRGs and the spinal cord is very limited, we will focus on intracellular cGMP signalling in this review.

Pathways involving cGMP are increasingly recognised as potential drug targets. In addition to already existing drugs that target cGMP pathways, such as NO-releasing nitrates (e.g., **glycerol trinitrate**,

molsidomine), stimulators of NO-GC and pGC (e.g., **riociguat**, **linaclotide**) or inhibitors of cGMP-hydrolysing PDEs (e.g., PDE3A/B inhibitors such as **milrinone** and **cilostazol**; PDE5A inhibitors such as **sildenafil** and tadalafil), new pharmacological approaches targeting cGMP-dependent pathways for the treatment of various diseases are being developed (Friebe et al., 2020). It seems possible that cGMP pathways in the somatosensory system might be the basis for future therapy of pain and itch. However, the functions of cGMP signalling during embryonic development and the wide tissue distribution of cGMP-related targets need to be considered to estimate the risk profile of novel drugs.

2 | DEVELOPMENTAL FUNCTION OF CGMP SIGNALLING IN SOMATOSENSORY NEURONS

The neuronal circuitry that provides the basic framework for transmission and processing of information in the somatosensory nervous system is laid down in a step-wise manner during embryonic and early postnatal development. The navigation of somatosensory axons that proceeds via a series of intermediate choice-points towards their target fields is controlled by coordinated action of membrane-associated and diffusible guidance cues with either permissive/attractive or inhibitory/repulsive properties (Chédotal, 2019). The activation of specific guidance receptors that are present on the growth cone—a highly motile structure at the tip of elongating neurites—triggers intracellular signalling cascades that regulate cytoskeletal rearrangements and membrane trafficking to generate directionality. Finally, the initial pattern of synaptic contacts is gradually refined by the pruning of electrically silent connections to establish the mature pattern of neuronal connectivity.

2.1 | A cross-talk between cGMP and signalling pathways activated by guidance cues

Many in vitro studies have demonstrated a role for cGMP in axonal growth and guidance of somatosensory neurons (Piper et al., 2007). For example, application of the membrane-permeable cGMP analogue, 8-Bromo-cGMP (8-Br-cGMP), to the culture medium exerted a growth-promoting effect on neonatal rat DRG neurons. Furthermore, neurites of DRG neurons from embryonic chicks and neonatal rats quickly re-oriented towards a point source of cGMP or 8-Br-cGMP, respectively (Gundersen & Barrett, 1980; Murray et al., 2009). These studies suggested that an asymmetrical distribution of cGMP across the extending growth cone promotes a directionally polarised extension. The application of cGMP analogues in in vitro assays that are used to study the effects of guidance cues (such as the growth cone turning assay, the stripe assay or the growth cone collapse assay) revealed that cGMP signalling affects the cellular response to specific guidance molecules. The first indication for such a modulatory role of cGMP (and also cAMP) derived from growth cone turning assays using *Xenopus* spinal neurons (Song et al., 1998). There, an increase in the concentration of cGMP facilitated the conversion of the repulsive effect of **semaphorin 3A** into an attractive one, whereas the attraction towards a point source of **neurotrophin-3** was converted into a repulsive response by inhibition of cGMP-dependent pathways using the PKG inhibitor Rp-cGMPS. Furthermore, an elevation of cGMP levels could counteract the collapse-inducing activity of semaphorin 3A in embryonic rat and mouse DRG neurons (Schmidt et al., 2002; Song et al., 1998). The response of axons of embryonic rat DRG neurons to the chemorepellent Slit2 could also be modulated in a cGMP-dependent manner (Nguyen-Ba-Charvet et al., 2001).

The initial conclusion that cGMP levels in the growth cone tune the response to the guidance cues semaphorin 3A, neurotrophin-3 and Slit2 (Song & Poo, 1999) was later refined to a concept in which the ratio of cAMP and cGMP levels, rather than their absolute concentrations, determines the cellular reaction to a specific guidance cue. The analysis of *Xenopus* spinal neurons' responses to the guidance factor netrin-1 in a turning assay revealed that a high cAMP/cGMP ratio favoured attraction via the netrin-1 receptor, also called 'deleted in colorectal cancer' (DCC), whereas an increase in the cGMP/cAMP ratio by application of 20 μM 8-Br-cGMP caused repulsion (Nishiyama et al., 2003). In contrast, activation of the netrin-1 receptor complex consisting of DCC and Unc5 led to rise in cGMP and a decrease in the ratio of cAMP/cGMP which resulted in repulsion. The antagonistic effects of cAMP and cGMP have been attributed to their reverse regulation of Ca^{2+} transients in the growth cone (Averaimo & Nicol, 2014). cAMP signalling via PKA facilitates Ca^{2+}-induced Ca^{2+} release from internal stores by activation of **ryanodine receptors** (Ooashi et al., 2005). In contrast, cGMP signalling via PKG1 leads to a reduction of ryanodine receptor-mediated Ca^{2+} release (Tojima et al., 2009). An inverse correlation of cAMP and cGMP levels has been observed in embryonic rat DRG axons by simultaneous FRET-imaging of both cyclic nucleotides (Kobayashi et al., 2013). PDEs like PDE2A and PDE3A/B whose cAMP-hydrolysing activity is either stimulated or inhibited by cGMP might provide a possible mechanism for a cross-talk between cGMP and cAMP (Zaccolo & Movsesian, 2007). In embryonic DRG, the reciprocal regulation of cGMP and cAMP might be mediated by PDE2A (Schmidt et al., 2016). Further intracellular effectors of cGMP signalling implicated in axonal guidance include components of the microtubule cytoskeleton regulating membrane organelle transport and CNG channels (Akiyama et al., 2016; Togashi et al., 2008). However, the detailed molecular mechanisms still need to be elucidated.

2.2 | A cGMP signalling cascade controls somatosensory axon bifurcation

During embryonic development, extensive axonal arborisation generates a further layer of complexity in the process of neuronal wiring. Axonal branching enables an individual neuron to link with different targets, an effect which provides the basis for distribution of information as well as cognitive integration. Impairments of axonal branching may result in severe neurological disorders (Chédotal, 2019; Nugent

et al., 2012). Despite its fundamental relevance for the establishment of neuronal connectivity, we are only beginning to understand key aspects of axonal branching including the identity of molecular signals and underlying mechanisms (Armijo-Weingart & Gallo, 2017; Kalil & Dent, 2014).

New branches can form either by splitting of the growth cone (mostly a bifurcation) or by budding of collaterals from the stem axon which is also known as interstitial branching (O'Leary et al., 1990). Both modes of axon branching can be observed in the afferent projections of somatosensory neurons into the spinal cord or hindbrain. In the mouse, afferent axons of DRG neurons reach the dorsal root entry zone (DREZ), the interface between the peripheral and the central nervous systems, during a developmental period between embryonic day (E) 10.5 and E13.5 (Figure 2a). The incoming axons then bifurcate into caudal and rostral branches that extend over several segments along the dorsolateral margin of the cord within the oval bundle of His. After a waiting period of at least 2 days, collateral branches emerge from the longitudinal stem axons and start to extend ventrally into the spinal cord. Collaterals from cutaneous neurons terminate in specific layers of the dorsal horn where they branch again and synapse onto second-order neurons. In contrast, the collaterals from proprioceptive neurons extend to more ventral regions of the spinal cord where terminal branching occurs and synaptic contacts with interneurons or motor neurons are formed (Brown, 1981; Chédotal, 2019; Ozaki & Snider, 1997).

The stereotypical branching pattern of primary sensory axon trajectories into the spinal cord can be easily visualised by tracing of lipophilic dyes or by genetic sparse labelling using specific mouse reporter lines (Schmidt et al., 2013; Schmidt & Rathjen, 2011), thereby generating an instrumental system for the investigation of molecular determinants of axon branching. So far, three components of a cGMP-dependent signalling cascade that regulates bifurcation of DRG axons in vivo have been identified (Schmidt et al., 2002, 2007, 2009; Zhao, Cao, et al., 2009; Zhao & Ma, 2009). The ligand **C-type natriuretic peptide** (CNP; also termed Nppc) is released from cells in the dorsal spinal cord. Binding of CNP to its receptor GC-B (also termed Npr2), on incoming axons of DRG neurons, triggers the intracellular production of the second messenger cGMP from GTP. Consequently, the α-isoform of PKG1—a serine/threonine kinase that is co-expressed with GC-B in all DRG neurons—becomes activated (Figure 2b). Based on the analysis of *lacZ*-reporter mice for CNP and GC-B, the expression of the ligand CNP starts at E9 forming a rostro-caudal gradient in the developing hind brain and spinal cord that slightly precedes that of its receptor GC-B in CSG and DRG (Schmidt et al., 2007; Schmidt et al., 2009). Transcriptional activity of GC-B in CSG and DRG begins between E9.5 and E10.5, thereby also forming a rostro-caudal gradient. All CSG and DRG are positive for GC-B by E11.5, and mRNA transcripts of the α-variant of PKG1 have been detected in mouse DRG at E10.5 by in situ hybridisation (Zhao, Cao, et al., 2009).

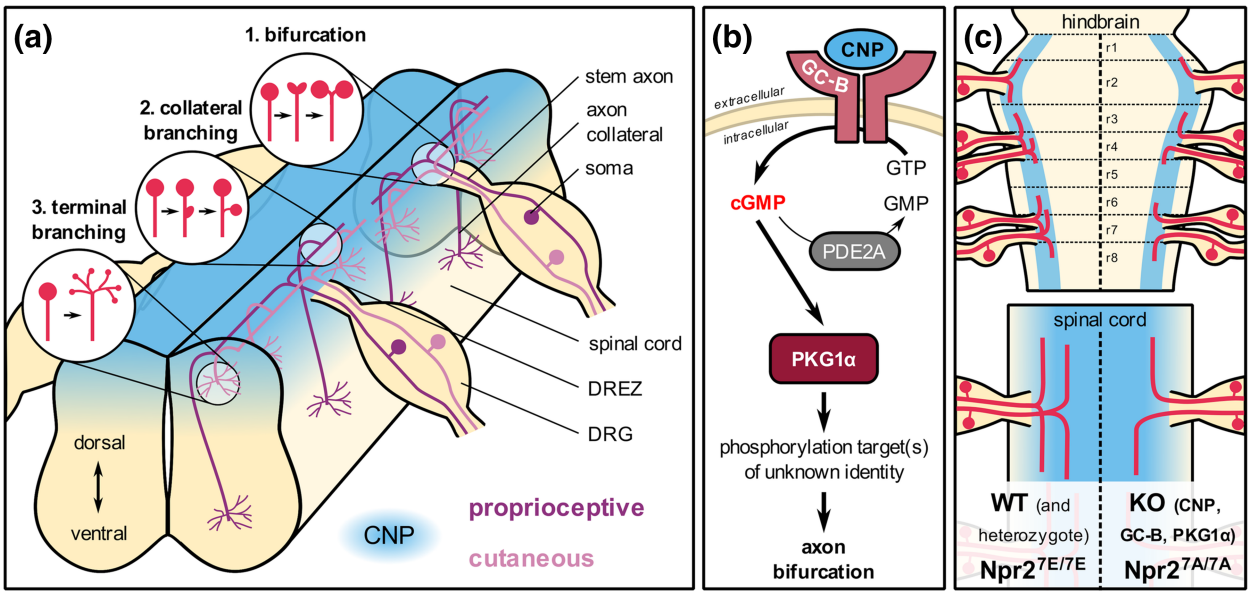

FIGURE 2 CNP-induced cGMP signalling regulates axon bifurcation in somatosensory neurons during embryonic development. (a) The axonal trajectories of DRG neurons into the embryonic spinal cord display different branching modes: 1. bifurcation at the DREZ, 2. collateral branching from stem axons and 3. terminal branching of cutaneous and proprioceptive axons in their dorsal and ventral termination zones, respectively. (b) The cGMP signalling pathway that controls somatosensory axon bifurcation includes the secreted ligand CNP, the receptor guanylyl cyclase GC-B and the kinase PKG1α whose targets remain to be identified. Degradation of cGMP in embryonic DRG neurons is predominantly catalysed by PDE2A. (c) Without CNP, GC-B or PKG1, somatosensory axon bifurcation is impaired in the hindbrain and in the spinal cord. Consequently, somatosensory axons turn either in an ascending or descending direction only. Axon bifurcation is also disabled in *Npr2*$^{7A/7A}$ mouse mutants lacking the phosphorylation of GC-B, whereas no differences are found between wildtype and *Npr2*$^{7E/7E}$ mutants expressing a phosphomimetic variant of GC-B. CNP, C-type natriuretic peptide; DREZ, dorsal root entry zone; DRG, dorsal root ganglia; GC-B, guanylyl cyclase B; KO, knock out; PDE2A, phosphodiesterase 2A; PKG1, protein kinase G 1; r1–8, rhombomeres 1–8; WT, wildtype

In mouse mutants deficient for either CNP, GC-B or PKG1, the afferent axons of DRG neurons fail to bifurcate and thus only turn either in a rostral or caudal direction (Figure 2c). The formation of collateral branches from the stem axons was not impaired in these mutants, indicating that axon bifurcation and interstitial branching might be controlled by distinct molecular mechanisms. However, the quantitative reduction of collateral branches due to the loss of longitudinal stem axons resulted in a reduced synaptic input on secondary neurons in electrophysiological recordings on CNP and GC-B mutant mice, whereas synaptic transmission per se was not (Schmidt et al., 2007, 2009). In addition to the loss of axon bifurcation, a small number of DRG axons prematurely entered the spinal cord in mice lacking CNP, GC-B or PKG1. The latter phenotype is reminiscent of observations in mice mutant for the guidance cues netrin-1, Slit1 and Slit2 or their receptors Unc5, Robo1 and Robo2 (Ma & Tessier-Lavigne, 2007; Watanabe et al., 2006). This might indicate that the dysregulation of cGMP signalling in the growth cones of sensory axons partly impedes the interpretation of repulsive guidance signals at the DREZ (Schmidt et al., 2002, 2007, 2009). However, it remains to be resolved how impaired cGMP signalling could result in a lack of sensitivity to repellent cues whereas otherwise cGMP has been demonstrated to exert growth promoting as well as anti-repellent and attractant effects. These apparently contradictory findings might reflect the use of different animal models and experimental assays as well as an involvement of different activators, generators and effectors of cGMP signalling, a varying degree of cross-talk with other signalling molecules or a differential compartmentalisation of those components in neurons.

A recent study using $Npr2^{fl/fl}$;$Wnt1^{Cre}$ mice, in which GC-B was selectively inactivated in DRG neurons, demonstrated the preservation of the gross anatomical structure of the spinal cord despite a complete loss of sensory axon bifurcation (Tröster et al., 2018). However, a detailed analysis of the three-dimensional shape of sensory axon projections within the spinal cord revealed a narrowing in their mediolateral extent as well as an increase in the dorsoventral span of collateral extensions in the absence of axon bifurcation. Again, these observations might be explained by altered terminal branching due to a reduced number of axon collaterals that is the consequence of the distorted bifurcation of stem axons. For a description of the functional consequences of conditional inactivation of GC-B in DRG neurons on pain processing the reader is referred to Section 3.2.

Activation of the cGMP-synthesising guanylyl cyclase domain of GC-B upon binding of CNP is critically dependent on the phosphorylation of seven serine and threonine residues within the regulatory kinase homology domain of the receptor (Potter & Hunter, 1998; Yoder et al., 2010, 2012). cGMP/FRET-imaging of cultivated DRG neurons from a mouse mutant, in which the seven serine and threonine residues in the kinase homology domain of GC-B were substituted by alanines ($Npr2^{7A/7A}$) to prevent phosphorylation (Figure 3), abolished CNP-induced cGMP signals. Consequently, axon bifurcation of somatosensory neurons was lost in the $Npr2^{7A/7A}$ mutant (Figure 2c), resembling the phenotype of CNP, GC-B and PKG1 global knockout mice (Schmidt et al., 2018). Interestingly, no alterations in somatosensory axon bifurcation were observed in a mouse mutant in which the seven identified serine and threonine residues were replaced by glutamic acid ($Npr2^{7E/7E}$) to generate a phosphomimetic variant of GC-B (Figures 2c and 3). The signalling pathways responsible for phosphorylation/dephosphorylation of GC-B in somatosensory neurons require further characterisation. Previously, an intracellular pathway comprising the small GTPase **Rac** and the **p21-activated kinase (PAK)** had been suggested to directly activate transmembrane guanylyl cyclases (Guo et al., 2007). Two recent studies indicated that at least in chondrocytes phosphorylation of GC-B might be linked to **FGFR3** signalling (Robinson et al., 2017; Shuhaibar et al., 2017).

Unlike GC-B, the cGMP-dependent regulation of sensory axon bifurcation does not involve NO-GCs. At the time of sensory axon bifurcation, NO-GC subunits are not expressed in embryonic mouse DRG neurons (Schmidt et al., 2007). Accordingly, recent

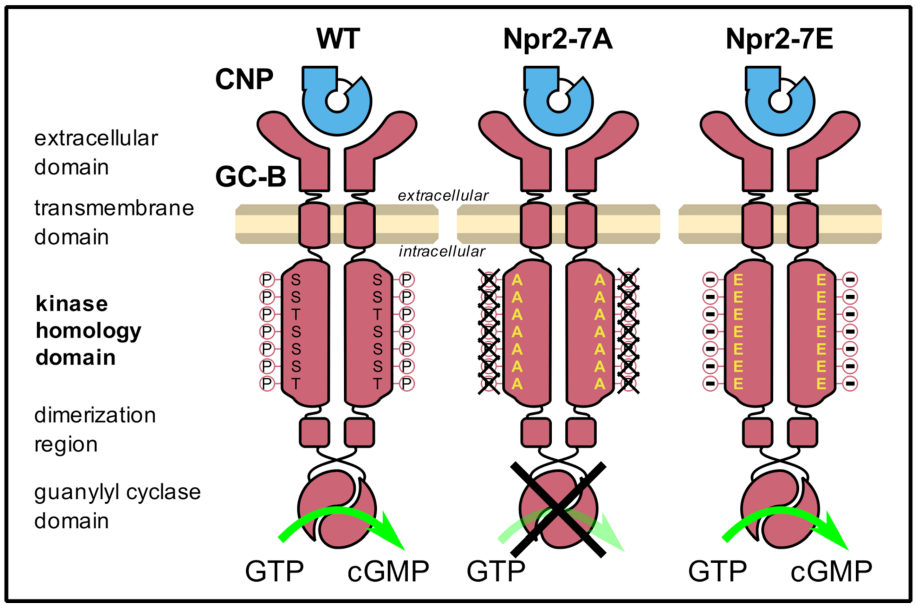

FIGURE 3 The generation of cGMP from GTP upon binding of CNP depends on the phosphorylation status of seven regulatory serine/threonine residues within the juxtamembrane GC-B kinase homology domain. A non-phosphorylatable version of GC-B (Npr2-7A) shows no guanylyl cyclase activity. In contrast, a mutant form of GC-B in which the substitution of the regulatory serine/threonine residues by glutamic acid mimics permanent phosphorylation (Npr2-7E), produces cGMP similar to the wildtype. A, alanine; CNP, C-type natriuretic peptide; E, glutamic acid; GC-B, guanylyl cyclase B; P, phosphorylation; S, serine; T, threonine; WT, wildtype

cGMP/FRET-imaging of dissociated embryonic DRG neurons expressing a genetically encoded cGMP-sensor demonstrated that in contrast to CNP, application of the NO donor DEA/NO did not result in a rise of cGMP levels (Schmidt et al., 2018). Moreover, no alterations in axon bifurcation and interstitial branching were observed in mouse mutants devoid of NO-induced cGMP signals due to the targeted inactivation of the β_1-subunit of NO-GC which is the common dimerizing subunit of NO-GCs (Schmidt et al., 2009).

The importance of cGMP for growth cone bifurcation requires a tight control of its intracellular concentration. Among several cGMP-degrading PDEs that have been detected at the mRNA level in embryonic DRG, PDE2A has been recently identified as the first functional PDE that hydrolyses CNP-induced cGMP in embryonic DRG neurons (Schmidt et al., 2016). In general, the dual specificity of cGMP-activated PDE2A for cAMP and cGMP enables a negative cross-talk between cGMP and cAMP signalling pathways (Bender & Beavo, 2006). cGMP/FRET measurements of dissociated DRG neurons from a cGMP sensor mouse line (Thunemann et al., 2013) revealed that only inhibitors specific for PDE2A led to an increase in CNP-induced cGMP similar to that observed after the application of the non-selective PDE inhibitor **IBMX**. In line with this observation, stimulation of embryonic DRG from PDE2A-deficient mice with CNP led to a highly increased level of cGMP as compared to DRG from wildtype mice, indicating that the loss of PDE2A activity was not compensated for by other PDE(s). However, mice deficient for PDE2A revealed no alterations in axon bifurcation, suggesting that sensory axon bifurcation is insensitive to increased cGMP levels (Schmidt et al., 2016). This surprising finding might indicate a role for other PDEs with distinct compartmentalisation in the control of cGMP levels during sensory axon bifurcation. Alternatively, the downstream mediator(s) of growth cone bifurcation after initial activation might become rapidly silenced again, keeping them unresponsive to a prolonged increase of cGMP levels, thereby ensuring that sensory axons bifurcate only once at the DREZ.

Analogous to DRG neurons, the CNP/GC-B/cGMP/PKG1α signalling pathway also controls the bifurcation of central axons of CSG neurons at the embryonic hindbrain (Figure 2c) as well as the bifurcation of axons from mesencephalic trigeminal neurons (MTN; also termed MesV) in rhombomere 2 at the level of the trigeminus. At embryonic stages both CSG and MTN neurons co-express GC-B and PKG1α, whereas at early stages of development CNP is distributed in a complementary fashion in rhombomeres 2, 4 and 6 at the entry zones of sensory afferents in the hindbrain. Tracing of individual CSG or MTN afferent axons in GC-B mutant mice demonstrated a lack of axon bifurcation in both neuronal subpopulations (Lu et al., 2011; Lu et al., 2014; Schmidt & Fritzsch, 2019; Ter-Avetisyan et al., 2014; Ter-Avetisyan et al., 2018). For a summary of the functional consequences of impaired axon bifurcation in spiral ganglion neurons on auditory function the reader is referred to the article by Marlies Knipper and Lukas Rüttiger on the 'Role of GCs for hearing function' in the same issue of BJP.

Moreover, MTN neurons are proprioceptive neurons which innervate muscles of mastication to relay information about jaw closing. They represent the only population of primary sensory neurons whose cell bodies reside within the CNS. The selective inactivation of GC-B-mediated cGMP signalling in MTN neurons in *Npr2*$^{fl/fl}$;*Engr1*^{Cre} mice resulted in the loss of axon bifurcation of MTN neurons in the brainstem while preserving bifurcation of trigeminal neurons. The comparison of *Npr2*$^{fl/fl}$;*Engr1*^{Cre} mice with *Npr2*$^{fl/fl}$ littermate controls showed a reduction of maximal biting forces indicating that the sensory feedback from jaw closing muscles might be impaired in the absence of MTN axon bifurcation in rhombomere 2 (Ter-Avetisyan et al., 2018).

Although numerous studies demonstrated the importance of CNP/GC-B/cGMP/PKG1α signalling for axon bifurcation, the downstream mechanisms remain poorly understood. In particular, the identity of the phosphorylation substrate(s) of PKG1α that mediate(s) axon bifurcation in somatosensory neurons in vivo remains to be determined. In vitro studies suggested that the regulation of axonal branching in DRG neurons by cGMP signalling might be mediated via the PKG1-dependent phosphorylation of **glycogen synthase kinase 3** (GSK3) (Zhao, Wang, et al., 2009), via the modification of microtubule dynamics (Akiyama et al., 2016; Xia et al., 2013) or by S-palmitoylation (Dumoulin et al., 2018). The application of CNP or cGMP analogues to cultures of DRG neurons elicited a PKG1-dependent increase in neurite length, growth cone area and the number of branching points (Dumoulin et al., 2018; Zhao & Ma, 2009; Zhao, Wang, et al., 2009). Furthermore, CNP has been proposed to exert an attractive effect on growth cone turning of embryonic rat DRG neurons (Zhao & Ma, 2009). However, a surge of growth cone bifurcation upon treatment with CNP could not be observed in these assays, indicating that the in vivo regulation of axon bifurcation could not be easily reproduced in vitro. Therefore, the elucidation of PKG1 downstream targets during cGMP-controlled axon bifurcation, for example using genetic mouse models, will be an important step in further research.

3 | CGMP SIGNALLING IN THE POSTNATAL SOMATOSENSORY SYSTEM

In addition to the intrinsically driven activity of sensory circuits during embryonic development, the nervous system continues to develop after birth. Maturation of somatosensory pathways gradually occurs throughout the early postnatal period (Chang et al., 2016; Fitzgerald, 2005), and aberrant sensory input caused by injury or inflammation during critical periods of early postnatal development can have long-term, detrimental effects on somatosensory processing (see Brewer & Baccei, 2020; Walker et al., 2016). Due to the marked effects of cGMP in embryonic development described in Section 2, it is possible that cGMP signalling also contributes to maturation of somatosensory pathways in the early postnatal period. However, the role of cGMP in this context remains elusive.

In the mature somatosensory system, the discrimination between various types of stimuli is achieved by populations of specialised sensory neurons that respond to specific stimuli and by numerous subtypes of connected neurons in the dorsal horn of the

spinal cord and at supraspinal sites (Emery & Ernfors, 2020; Häring et al., 2018; Zeisel et al., 2018). There is accumulating evidence that, in the mature somatosensory system, multiple cGMP-dependent signalling pathways exist that are involved in a variety of sensory processes. It becomes more and more clear that cGMP is produced by different sources in distinct populations of primary afferent and spinal cord neurons and in peripheral glial cells. The resulting cGMP effects are presumably mediated by various downstream effectors.

3.1 | GC-A signalling plays a pivotal role in the processing of itch

In the past few years, substantial progress has been made in understanding the mechanisms underlying the sensation of itch. In general, itch (also known as pruritus) is an uncomfortable experience that evokes a desire to scratch. Although acute itching is a protective reaction that assists in the removal of irritants and calls attention to the affected skin areas, chronic itch is a major concern, particularly because most available treatments are only partly effective. Conditions associated with chronic itch include, for example, dermatological conditions such as psoriasis and atopic dermatitis, systemic diseases such as renal and liver failure, neurological diseases and psychiatric disorders (Dong & Dong, 2018). Among the itch-sensing sensory neurons is a population that contains high levels of **B-type natriuretic peptide** (BNP; also called Nppb). Of note, single-cell RNA-sequencing data of the peripheral and central nervous systems in mice indicate that BNP is nearly exclusively localised to this population of neurons in the peripheral nervous system (Zeisel et al., 2018). This new finding challenges a previous hypothesis that BNP might be highly expressed in CNS neurons, which was based on its initial discovery in porcine brain extracts and led to the misleading name 'brain natriuretic peptide' (Sudoh et al., 1988).

The BNP-positive population of sensory neurons contains several receptors of itch mediators such as the **IL-31 receptor α**, 5-HT receptors (**5-HT$_{1A}$**, **5-HT$_{1F}$** and **5-HT$_{2A}$**) and histamine **H$_1$ receptor** (Dong & Dong, 2018; Meng et al., 2020; Solinski et al., 2019; Usoskin et al., 2015). The release of **IL-31**, **5-HT**, **histamine** and additional itch mediators from mast cells or keratinocytes is believed to stimulate the respective itch receptors on BNP-positive sensory neurons. As a consequence, BNP is released from the central endings of these neurons, which terminate in lamina II of the spinal dorsal horn. BNP then activates its receptor GC-A (also termed Npr1), which is expressed in a population of spinal interneurons (Mishra & Hoon, 2013). The activation of GC-A-positive neurons induces the release of another neuropeptide, **gastrin-releasing peptide (GRP)**, which relays the signal to a group of interneurons that express the receptors for GRP, known as **BB$_2$ receptors**, an essential player of itch processing in the spinal cord. These neurons then transmit the signal to the brain so that itch is ultimately perceived as a major sensation that is distinguishable from pain and others (Dumoulin et al., 2018; Huang et al., 2018; Liu et al., 2021; Pagani et al., 2019; Sun et al., 2009).

The functional relevance of BNP/GC-A signalling for itch processing is reflected by the observation that intrathecal injection of BNP in mice elicits scratching behaviour and that mice lacking BNP (*Nppb*$^{-/-}$) exhibited greatly attenuated responses to a range of pruritic agents. By contrast, *Nppb*$^{-/-}$ mice had unaltered pain responses to noxious thermal and mechanical stimuli as well as in models of persistent pain, suggesting an itch-specific function of BNP (Mishra & Hoon, 2013; Pitake et al., 2017). Moreover, ablation of GC-A-positive dorsal horn neurons by intrathecal administration of a BNP-saporin conjugate and/or genetic knockout of GC-A attenuated histamine-induced pruritus and chronic itch in a model of allergic contact dermatitis (Huang et al., 2019; Liu et al., 2021; Mishra & Hoon, 2013). Although cGMP levels were not measured in these studies, it is likely that cGMP signalling in GC-A-positive dorsal horn neurons mediates the downstream effects. Further studies are needed to confirm the production of cGMP in dorsal horn neurons, to clarify the functional relevance of cGMP in itch processing, to identify targets that mediate the cGMP effects and to elucidate which PDEs degrade cGMP in these neurons.

In addition to the spinal cord, BNP/GC-A signalling has recently been found to drive peripheral inflammation in different forms of dermatitis, thereby enhancing pruritus. In particular, BNP and GC-A expression has been reported to be upregulated during chronic itch and suggested to orchestrate cytokine and chemokine release from skin cells (Meng et al., 2018). Hence, it is tempting to speculate that BNP/GC-A/cGMP signalling affects itch also by peripheral mechanisms. A detailed description of peripheral and central BNP/GC-A/cGMP signalling in chronic itch is provided in a recently published review (Meng et al., 2020). Interestingly, in a recent study a novel low MW inhibitor of GC-A, JS-11, was not only effective in attenuating acute itch but also reduced scratching in a model for persistent inflammatory dermatitis that is associated with chronic itch (Solinski et al., 2019). Hence, the BNP/GC-A axis could be a useful target for novel anti-itch therapies (Otto, 2019).

3.2 | Emerging evidence for GC-B signalling in the processing of pain

In contrast to the essential role of CNP/GC-B signalling for axonal branching of sensory neurons in embryonic development (see Section 2), the somatosensory functions of CNP and GC-B during adulthood are only poorly understood. In an earlier study, we observed that intrathecal injection of CNP into the lumbar spinal cord region of adult mice led to a hindpaw hypersensitivity that was antagonised by a PKG1 inhibitor (Schmidtko et al., 2008), suggesting that CNP contributes to pain sensitisation in a cGMP- and PKG1-dependent manner. Another study reported that intraplantar injection of CNP into the hindpaw of adult mice induced a thermal hyperalgesia that persisted over several hours (Loo et al., 2012). In accordance with this finding, patch clamp experiments revealed that CNP can potentiate capsaicin- and proton-activated currents of cultured DRG neurons and increase their firing frequency. In the same study, a non-canonical

G-protein-dependent signalling pathway mediated by the natriuretic peptide clearance receptor **NPR-C** was supposed to mediate these pro-nociceptive effects of CNP (Loo et al., 2012). However, this hypothesis was later challenged by single-cell RNA sequencing studies in DRG neurons from adolescent and adult mice, which detected NPR-C expression only at low levels and in a minority of DRG neuron populations (Sharma et al., 2020;Usoskin et al., 2015; Zeisel et al., 2018).

Although direct evidence is missing, the most likely downstream mediator of CNP in the mature somatosensory system is, as in embryonic development, GC-B. Expression of GC-B in many populations of DRG neurons of adolescent or adult rodents has been consistently detected by immunostaining (Abdelalim et al., 2013; Schmidtko et al., 2008) and RNA sequencing analyses (Usoskin et al., 2015; Zeisel et al., 2018), albeit the GC-B expression levels in adult stages are considerably lower than during embryonic development (Tröster et al., 2018). In a recent study in mice lacking GC-B in sensory neurons (*Npr2*$^{fl/fl}$;*Wnt1*^{Cre}; see Section 2.2) we found that the responses to acute noxious heat stimuli, which were elicited by placing the animals on a hot plate or stimulating the paws with a radiant heat source, were attenuated (Tröster et al., 2018). Furthermore, these animals showed reduced nocifensive responses to paw injection of chemical irritants such as capsaicin or formalin (Tröster et al., 2018), pointing to an involvement of GC-B in the processing of acute nociceptive pain. However, as *Npr2*$^{fl/fl}$;*Wnt1*^{Cre} mice exhibit impaired sensory axon bifurcation (see Section 2.2) it is not clear whether their attenuated pain responses are caused by the lack of GC-B-mediated cGMP signalling, the bifurcation deficits or both.

Another aspect that awaits further analysis is the relevance of CNP/GC-B/cGMP signalling for neurite outgrowth and regeneration of sensory neurons in adulthood. The cGMP analogue 8-Br-cGMP has been shown to promote neurite outgrowth in a PC12 neuronal cell line (Yamazaki et al., 2004) and in cultured DRG neurons of adult rats (Murray et al., 2009). Although these data point to a contribution of cGMP to neurite outgrowth in adulthood, it remains to be elucidated whether CNP and GC-B are also involved, as in embryonic development (see Section 2). Further studies, for example in mice without developmental defects, are needed to elucidate the effects of CNP/GC-B/cGMP signalling on the processing of somatosensory stimuli and regeneration of injured peripheral nerves in adulthood and to unravel potential upstream pathways and downstream effectors.

3.3 | Dual function of NO-GC signalling during persistent pain

Abundant evidence over the past three decades indicates an important role of NO-dependent cGMP production in the processing of chronic pain. For example, administration of a range of inhibitors of NO synthases (NOS) or NO-GCs ameliorated the pain behaviour of mice and rats in various models of persistent inflammatory and neuropathic pain (Luo & Cizkova, 2000). Accordingly, genetic knockout of ***NOS1*** (which encodes for neuronal NOS, nNOS) or the essential β_1-subunit of NO-GC attenuated inflammatory and neuropathic pain (Chu et al., 2005; Guan et al., 2007; Schmidtko et al., 2008). These earlier studies have been summarised in previously published comprehensive reviews (Schmidtko, 2015; Schmidtko et al., 2009).

Recent work has increased our understanding about the areas of NO/cGMP action in the somatosensory system. Among the three NOS isoforms, nNOS seems to be the most relevant in pain processing (Schmidtko, 2015). In the spinal cord, nNOS is expressed in a population of neurons that are scattered throughout the dorsal horn with enrichment in laminae IIi to III (Todd, 2017). A histochemical classification suggests that these nNOS-positive cells constitute one of five largely non-overlapping populations of inhibitory interneurons (in addition to cells positive for **galanin**, **neuropeptide Y**, calretinin or parvalbumin; Hughes & Todd, 2020). Accumulating evidence suggests that NO produced by these inhibitory interneurons is of particular relevance for the processing of persistent pain (Figure 4). For example, in accordance with the finding that intrathecally injected NOS inhibitors attenuated the pain behaviour in various models, immunostaining experiments revealed that NO-GC is localised in close proximity to nNOS-positive cells in the dorsal horn (Ding & Weinberg, 2006; Ruscheweyh et al., 2006; Schmidtko et al., 2008). Notably, in a recent study we found that the two NO-GC isoforms, GC-1 and GC-2, were both present in the spinal dorsal horn but showed different distribution patterns: Using immunostaining we observed that the area of prominent GC-1 expression is restricted to laminae IIi/v-IIIo, whereas prominent GC-2 expression is more broadly distributed in laminae IIi/v-IV of the dorsal horn. Moreover, in situ hybridisation experiments revealed that in the dorsal horn GC-1 is nearly exclusively expressed in inhibitory interneurons, whereas GC-2 expression also includes excitatory interneurons (Petersen et al., 2019). The localisation of NO-GC and nNOS in the dorsal horn and suggested NO/NO-GC/cGMP signalling pathways are summarised in Figure 4.

To assess the role of the NO-GC isoforms in pain processing in vivo, we analysed the pain behaviour of mice lacking GC-1 and GC-2 (*GC*$\alpha_1^{-/-}$ and *GC*$\alpha_2^{-/-}$, respectively). Interestingly, in two models of neuropathic pain induced by peripheral nerve injury, *GC*$\alpha_1^{-/-}$ mice demonstrated reduced hypersensitivity at late stages after the injury, whereas the behaviour of *GC*$\alpha_2^{-/-}$ mice was similar to that of WT littermates. A reduced neuropathic pain behaviour was also observed in tissue-specific *Lbx1-GC*$\alpha_1^{-/-}$ mice, in which GCα_1 is selectively deleted in dorsal horn neurons, confirming that signalling in the dorsal horn of the spinal cord underlays the altered neuropathic pain behaviour (Petersen et al., 2019). Opposite findings resulted from the analysis of these animals in models of inflammatory pain: *GC*$\alpha_1^{-/-}$ mice showed an unaltered pain behaviour, but the hyperalgesia of *GC*$\alpha_2^{-/-}$ mice was unexpectedly increased as compared to WT mice. Similarly, the tissue-specific knockout of GC-2 in *Lbx1-GC*$\alpha_2^{-/-}$ mice mimicked the increased inflammatory pain of global *GC*α*2*$^{-/-}$ mice (Petersen et al., 2019). Hence, GC-1 and GC-2 in the spinal dorsal horn exert strikingly different functions in the processing of neuropathic and inflammatory pain,

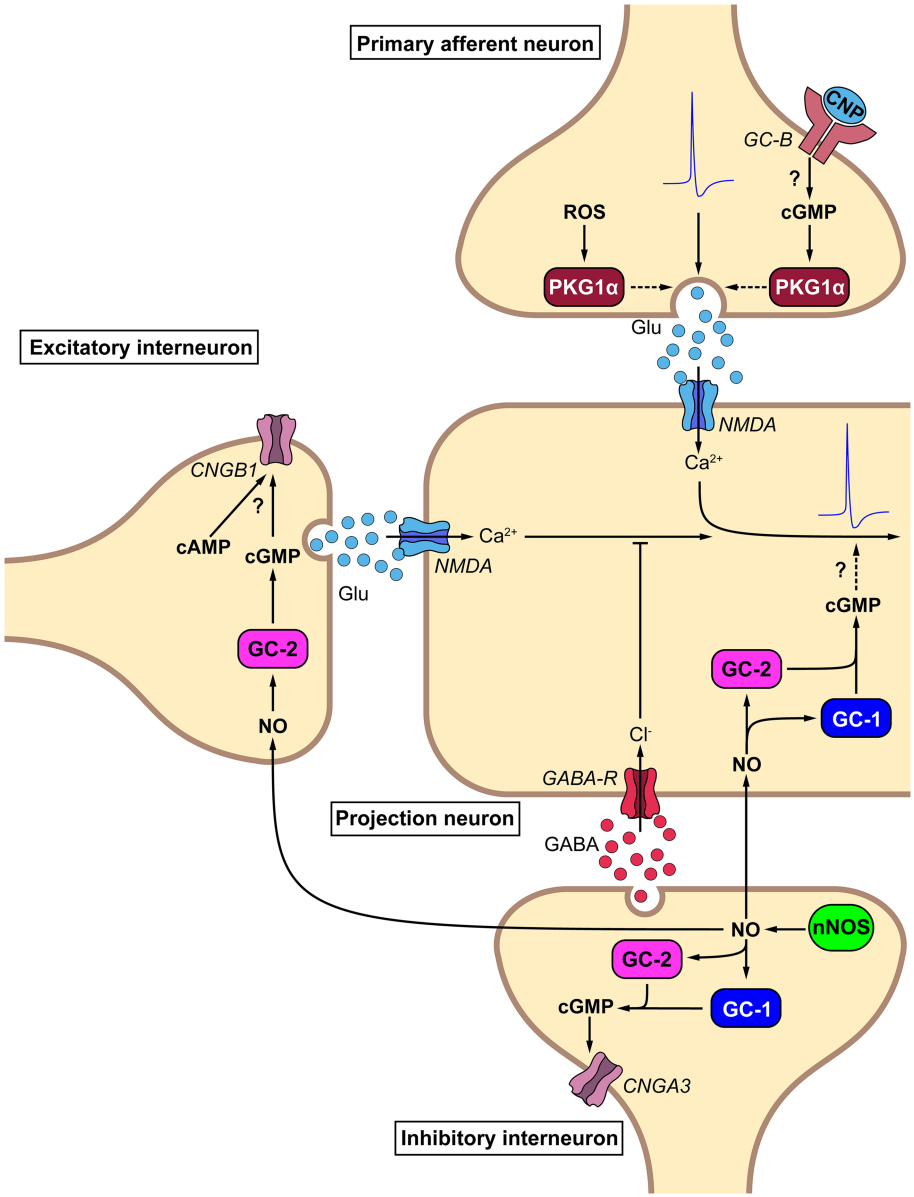

FIGURE 4 Suggested cGMP signalling pathways in the dorsal horn of the spinal cord in adulthood. nNOS is mainly localised to inhibitory interneurons. Both NO-GC isoforms are expressed by inhibitory interneurons and projection neurons, whereas GC-2 is also present in excitatory interneurons. cGMP produced by NO-GC activates CNGA3 in inhibitory interneurons, eventually CNGB1 in excitatory interneurons and so far unidentified further downstream targets. In contrast to NO-GC, PKG1α is mainly expressed in primary afferent neurons and activated by cGMP, which most likely derives from CNP-mediated activation of GC-B, and by ROS in a cGMP-independent manner. PKG1α has been shown to regulate the neurotransmitter release from sensory neurons. CNG, cyclic nucleotide-gated; CNP, C-type natriuretic peptide; GC-B, guanylyl cyclase B; nNOS, neuronal NO synthase; NO, nitric oxide; NO-GC, nitric oxide-sensitive guanylyl cyclase; PKG1α, protein kinase G 1α

suggesting that NO/cGMP signalling in the CNS is more complex than previously hypothesised (Meller & Gebhart, 1993). Furthermore, the expression of the two NO-GC isoforms in distinct populations of excitatory and inhibitory interneurons of the dorsal horn might be an explanation for the dual effects (both pronociceptive and antinociceptive) of NO donors and cGMP analogues reported in earlier studies (Schmidtko, 2015).

nNOS is also expressed in sensory neurons, albeit with a variable distribution in different DRG segments: Although up to 23% of DRG neurons from segments Th5 to L1 are nNOS-positive, less than 2% of DRG neurons from C1 to Th4 and from L2 through the sacral levels contain nNOS (Aimi et al., 1991). Given the fact that NO readily diffuses through plasma membranes, it is likely that NO/cGMP signalling in the peripheral nervous system affects somatosensory function. Importantly, the essential NO-GC subunit $GC\beta_1$, which contains the N-terminal HNOX (haem NO/oxygen binding) domain required for binding NO (Wedel et al., 1995) is not expressed in sensory neurons (Petersen et al., 2019; Schmidtko et al., 2008; Usoskin et al., 2015), thus excluding the possibility that GC-1 (subunit composition $GC\alpha_1/GC\beta_1$) or GC-2 (subunit composition $GC\alpha_2/GC\beta_1$) serve as NO receptor in sensory neurons. However, a functional NO-GC isoform (GC-2) is present in satellite glial cells that wrap around neuronal cell bodies of the peripheral nervous system (Petersen et al., 2019; Schmidtko et al., 2008). In line with this, exposure of acutely dissected DRGs to NO donors led to cGMP production exclusively in satellite glial cells (Thippeswamy & Morris, 2002). Interestingly, recent work revealed that NO donors and cGMP analogues increase gap junctional communications among satellite glial cells, which in turn might affect the excitability of DRG neurons (Belzer & Hanani, 2019). The effects of satellite glial cells on the somatosensory processes and neuronal homeostasis is still developing and has been discussed in detail in a recent review (Hanani & Spray, 2020), but the exact role of cGMP signalling in these cells remains to be elucidated.

Furthermore, the lack of NO-GC in sensory neurons challenges the conclusion of earlier studies that a NO-GC/cGMP/PKG1 signalling pathway leading to activation of ATP-sensitive potassium channels is functional in sensory neurons (Alves et al., 2013; Costa et al., 2014; de Carvalho Veloso et al., 2015; Sachs et al., 2004; Spiller et al., 2019). In these studies, inhibitors of NOS (such as **L-NAME**, **NOArg** or L-NPA), NO-GC (such as ODQ or methylene blue), PKG1 (such as KT5823) or ATP sensitive potassium channels (such as **glibenclamide**) inhibited the pain behaviour or reversed the antinociceptive effects of other drugs in rodents. It seems likely that the behavioural responses were partly mediated by targets outside sensory neurons and that different signalling pathways might be involved, including cGMP-independent NO signalling via S-nitrosylation (Chen et al., 2017) and cGMP-independent activation of PKG1 (see Section 3.4). Furthermore, the matter of selectivity of the used compounds needs to be taken into account.

3.4 | PKG1 is a major cGMP effector in sensory neurons

A major downstream effector of cGMP in the somatosensory system is PKG1 (Hofmann, 2020). Previous studies revealed that PKG1 is expressed in the vast majority of sensory neurons with high expression levels in nociceptors (Luo et al., 2012) (Figure 4). Pain-relevant functions of PKG1 have been well documented in a series of studies with animals lacking PKG1 specifically in **$Na_V1.8$**-positive nociceptors (*SNS-PKG1*$^{-/-}$ mice). In accordance with mice lacking PKG1 globally (Tegeder et al., 2004), *SNS-PKG1*$^{-/-}$ mice demonstrated marked behavioural defects in models of inflammatory pain (Luo et al., 2012). In particular, the hypersensitivity induced by complete Freund's adjuvant (CFA), capsaicin and acid and the licking behaviour in the formalin test were attenuated in *SNS-PKG1*$^{-/-}$ mice. Further work showed that activity-induced long-term potentiation at synapses between nociceptors and spinal projection neurons is abolished in *SNS-PKG1*$^{-/-}$ mice and that PKG1 regulates the probability of neurotransmitter release from sensory neurons (Luo et al., 2012). Another study indicates that neuronal activity in supraspinal regions along ascending pain pathways is reduced if PKG1 is absent from nociceptors (Gangadharan et al., 2017). Moreover, recent work using *SNS-PKG1*$^{-/-}$ mice suggested that PKG1 expressed in nociceptors is also involved in the processing of neuropathic pain after peripheral nerve injury and serves as a critical generator for central sensitisation and cortical plasticity (Wang et al., 2021). However, this finding contrasts with a previous study using the same pain model in which *SNS-PKG1*$^{-/-}$ mice developed stronger neuropathic hypersensitivity (Valek et al., 2017).

With regard to cGMP signalling it should be noted that activation of PKG1 can also be driven by cGMP-independent mechanisms (Figure 4). PKG1α, the major isoform of the enzyme in the cytosol of sensory neurons (Uchida et al., 2018), can also be activated by oxidants that are able to cause an interprotein disulfide bond formation between two cysteine residues (Cys^{42}) on adjacent chains in the PKG1α homodimer. This reaction renders the kinase catalytically active, independently of cGMP (Burgoyne et al., 2007). In a study in global knock-in mice lacking the redox-sensing Cys^{42} thiol (C42S PKGI-α knock-in mice; Prysyazhna et al., 2012), we found that this oxidant-dependent disulfide activation of PKG1α plays an important role for neuropathic pain hypersensitivity after peripheral nerve injury but has a limited contribution to inflammatory pain processing (Lorenz et al., 2014). This finding is in accordance with accumulating evidence that reactive oxygen species (ROS) such as superoxide anion or **hydrogen peroxide** are produced in the nerve and DRGs after peripheral nerve injury (Kallenborn-Gerhardt et al., 2012, 2013). Moreover, cultured DRG neurons from these knock-in mice demonstrated enhanced neurite outgrowth and reduced growth cone collapse in response to ROS stimulation (Valek et al., 2017), suggesting that cGMP-independent activation of PKG1 might also be involved in nerve repair after injury.

Based on the important role of PKG1 in pain sensitisation, novel inhibitors of PKG1 are being developed for treatment of chronic pain. Interestingly, the low MW compound N46, which inhibits PKG1 in the low nanomolar range and does not cross the blood–brain-barrier, attenuated the hypersensitivity induced by CFA or capsaicin and was effective in a model of osteoarthritic knee joint pain in rats (Qin et al., 2018; Sung et al., 2017). However, the safety profile of PKG1 inhibitors remains to be evaluated, because PKG1 is highly expressed in various tissues including the heart and intestine and plays an essential role during embryonic development of the somatosensory system (see Section 2).

By which mechanisms PKG1 mediates these effects in pain processing remains poorly understood. Proteins that have been reported to be phosphorylated by PKG1 in sensory neurons include cysteine-rich protein 4 (CRP4, also known as cysteine-rich protein 2; gene *Crip2*; Schmidtko et al., 2008), myosin light chain (αMLC), **inositol 1,4,5-trisphosphate receptor 1 (IP_3R1)** and vasodilator-stimulated phosphoprotein (VASP) (Luo et al., 2012). Given the high number of PKG1 substrates that have been identified in other tissues (Feil & Kleppisch, 2008) and the fact that many of these proteins are expressed in sensory neurons (Usoskin et al., 2015), it seems likely that PKG1 signalling during persistent pain involves more phosphorylation substrates than those known today. Moreover, it remains to be elucidated whether cGMP- and ROS-dependent activation of PKG1 is connected with different effector mechanisms.

3.5 | Somatosensory functions of CNG channels and PDEs

Beyond PKG, cGMP can also signal through **CNG channels**. These channels, which are activated by direct binding of cGMP or cAMP, play an essential role in visual and olfactory signal transduction (Biel & Michalakis, 2009; Michalakis et al., 2018) and are increasingly recognised as a cyclic nucleotide target in the CNS (Podda &

Grassi, 2014). In a previous study we observed that in response to paw inflammation, the expression of the CNG channel subunit **CNGA3** is up-regulated in inhibitory interneurons of the spinal dorsal horn and in DRG satellite cells. Furthermore, mice lacking CNGA3 showed increased inflammatory pain behaviours and exaggerated pain hypersensitivity after intrathecal delivery of NO donors and cGMP analogues (Heine et al., 2011). These data suggest that CNGA3-positive CNG channels are a downstream target of NO/cGMP signalling that contributes in an inhibitory manner to persistent pain processing. In a recent study we found that the subunit **CNGB1** plays a role in neuropathic pain after peripheral nerve injury (Kallenborn-Gerhardt et al., 2020). In particular, the CNGB1b subunit was detected in excitatory interneurons in the spinal dorsal horn (Figure 4) and in sensory neurons, and CNGB1 knockout (*CNGB1*$^{-/-}$) mice exhibited considerably attenuated neuropathic pain behaviours. However, the observation that hindpaw sensitivity after intrathecal administration of a cAMP analogue, but not of a cGMP analogue, was reduced in *CNGB1*$^{-/-}$ mice points to CNGB1 as a downstream effector of cAMP rather than cGMP in pain pathways (Kallenborn-Gerhardt et al., 2020).

There is a big gap in our knowledge about the PDEs that degrade cGMP in the adult somatosensory system. In a previous study we found that PDE2A expression in the spinal dorsal horn is up-regulated during paw inflammation and that intraperitoneal treatment with the selective PDE2A inhibitor **BAY 60-7550** increased the inflammatory pain hypersensitivity in mice. However, experiments with intrathecally injected cAMP and cGMP analogues and the detection of increased cAMP levels ex vivo point towards PDE2A being mainly connected to cAMP signalling in the spinal cord (Kallenborn-Gerhardt et al., 2014). Conversely, recent research revealed that intrathecal administration of BAY 60-7550 attenuated the mechanical allodynia in a model of lumbar disc herniation in rats and increased the levels of both cAMP and cGMP in spinal cord homogenates (Wang, Zhao, et al., 2017). About the reasons for these discrepancies we can only speculate, but model-dependent changes in PDE2A expression in the spinal cord and the different routes of drug administration in both studies might be an explanation.

Other studies have reported anti-nociceptive effects of PDE5A inhibitors, such as sildenafil, tadalafil and vardenafil, in various rodent pain models including diabetic neuropathy (Gediz et al., 2015; Jain et al., 2001; Otari & Upasani, 2015; Patil et al., 2004; Wang, Zhao, et al., 2017). Considering that single-cell RNA-sequencing analyses failed to detect significant PDE5A expression in somatosensory neurons (Usoskin et al., 2015; Zeisel et al., 2018), an altered vascular function might be one reason for the observed behavioural effects. This seems to be of importance in models of diabetic neuropathy, in which PDE5A inhibitors have been shown to improve blood supply to the vasa nervorum within peripheral nerves (Wang, Chopp, & Zhang, 2017). Another explanation could rely on off-target effects of sildenafil, tadalafil and vardenafil, which in addition to PDE5A also inhibit enzymes of the PDE1, PDE6 and PDE11 families in the nanomolar or low micromolar range (Gbekor et al., 2002). Hence, further studies are required to uncover the PDEs that control cGMP signalling in the adult somatosensory system.

4 | FUTURE DIRECTIONS

The recent advances in our understanding of cGMP signalling pathways in somatosensation will facilitate drug development. Of critical importance for a mechanistic understanding is the identification of PKG1α phosphorylation substrate(s), alternative cGMP targets and PDEs that terminate cGMP signalling and the analysis of spatiotemporal dynamics of cGMP production. Tools like genetically encoded cGMP/FRET sensors (Nikolaev et al., 2006; Thunemann et al., 2013), cGMP chelators (Ros et al., 2019) or controllable guanylyl cyclases for optogenetic manipulation of cGMP (Gao et al., 2015) will be instrumental for further elucidation of cGMP functions in the somatosensory system.

4.1 | Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to Pharmacology (http://www.guidetopharmacology.org) and are permanently archived in the Concise Guide to Pharmacology 2019/20 (Alexander, Christopoulos et al., 2019; Alexander, Fabbro, et al., 2019a, b; Alexander, Mathie, et al., 2019; Beuve et al., 2020; Billiar et al., 2019).

ACKNOWLEDGEMENTS

The authors disclosed receipt of the following financial support for the research, authorship and/or publication of this article: This work was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) with Research Unit FOR 2060 project SCHM 2371/1 to Hannes Schmidt, project 335549539/GRK2381 to Alexandra Böttcher and Hannes Schmidt and Research Unit FOR 2060 project SCHM 2629/3-1 and project SCHM 2629/4-1 to Achim Schmidtko. This article is based in part upon work from the DFG-funded Research Unit FOR 2060.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article because no new data were created or analysed in this study.

REFERENCES

Abdelalim, E. M., Bellier, J.-P., & Tooyama, I. (2013). Expression of NPR-B in neurons of the dorsal root ganglia of the rat. *Peptides*, *43*, 56–61. http://doi.org/10.1016/j.peptides.2013.02.013

Aimi, Y., Fujimura, M., Vincent, S. R., & Kimura, H. (1991). Localization of NADPH-diaphorase-containing neurons in sensory ganglia of the rat. *The Journal of Comparative Neurology*, *306*(3), 382–392. http://doi.org/10.1002/cne.903060303

Akiyama, H., Fukuda, T., Tojima, T., Nikolaev, V. O., & Kamiguchi, H. (2016). Cyclic nucleotide control of microtubule dynamics for axon guidance. *Journal of Neuroscience*, *36*(20), 5636–5649. http://doi.org/10.1523/jneurosci.3596-15.2016

Alexander, S. P. H., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharman, J. L., Southan, C., Davies, J. A., & Collaborators, C. G. T. P. (2019). The Concise Guide to PHARMACOLOGY 2019/20: G protein-coupled receptors. *British Journal of Pharmacology*, *176*, S21–S141. https://doi.org/10.1111/bph.14748

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharman, J. L., Southan, C., Davies, J. A., & CGTP Collaborators. (2019a). The Concise Guide to PHARMACOLOGY 2019/20: Catalytic receptors. *British Journal of Pharmacology*, *176* (Suppl 1), S247–S296. https://doi.org/10.1111/bph.14751

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharman, J. L., Southan, C., Davies, J. A., & CGTP Collaborators. (2019b). The Concise Guide to PHARMACOLOGY 2019/20: Enzymes. *British Journal of Pharmacology*, *176*(Suppl 1), S297–S396. https://doi.org/10.1111/bph.14752

Alexander, S. P. H., Mathie, A., Peters, J. A., Veale, E. L., Striessnig, J., Kelly, E., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharman, J. L., Southan, C., Davies, J. A., & CGTP Collaborators. (2019). The Concise Guide to PHARMACOLOGY 2019/20: Ion channels. *British Journal of Pharmacology*, *176*(Suppl 1), S142–S228. https://doi.org/10.1111/bph.14749

Alves, D. P., Da Motta, P. G., Romero, T. R. L., Klein, A., & Duarte, I. D. G. (2013). NO/cGMP production is important for the endogenous peripheral control of hyperalgesia during inflammation. *Nitric Oxide*, *28*, 8–13. https://doi.org/10.1016/j.niox.2012.09.001

Armijo-Weingart, L., & Gallo, G. (2017). It takes a village to raise a branch: Cellular mechanisms of the initiation of axon collateral branches. *Molecular and Cellular Neurosciences*, *84*, 36–47. https://doi.org/10.1016/j.mcn.2017.03.007

Aronsen, L., Orvoll, E., Lysaa, R., Ravna, A. W., & Sager, G. (2014). Modulation of high affinity ATP-dependent cyclic nucleotide transporters by specific and non-specific cyclic nucleotide phosphodiesterase inhibitors. *European Journal of Pharmacology*, *745*, 249–253. https://doi.org/10.1016/j.ejphar.2014.10.051

Averaimo, S., & Nicol, X. (2014). Intermingled cAMP, cGMP and calcium spatiotemporal dynamics in developing neuronal circuits. *Frontiers in Cellular Neuroscience*, *8*, 1–10. http://doi.org/10.3389/fncel.2014.00376

Belzer, V., & Hanani, M. (2019). Nitric oxide as a messenger between neurons and satellite glial cells in dorsal root ganglia. *Glia*, *67*(7), 1296–1307. https://doi.org/10.1002/glia.23603

Bender, A. T., & Beavo, J. A. (2006). Cyclic nucleotide phosphodiesterases: Molecular regulation to clinical use. *Pharmacological Reviews*, *58*(3), 488–520. https://doi.org/10.1124/pr.58.3.5

Beuve, A., Brouckaert, P., Burnett, J. J. C., Friebe, A., Garthwaite, J., Hobbs, A. J., Koesling, D., Kuhn, M., Potter, L. R., Russwurm, M., & Schmidt, H. H. (2020). Receptor guanylyl cyclase (RGC) family (version 2020.3) in the IUPHAR/BPS guide to Pharmacology database. *IUPHAR/BPS Guide to Pharmacology CITE*, *2020*(3), 1–4. https://doi.org/10.2218/gtopdb/F1022/2020.3

Biel, M., & Michalakis, S. (2009). Cyclic nucleotide-gated channels. In H. H. H. W. Schmidt & L. K. Antos (Eds.), Handbook of Experimental Pharmacology: Vol. 191. *cGMP: Generators, effectors and therapeutic implications* (pp. 111–136). Berlin Heidelberg: Springer. https://doi.org/10.1007/978-3-540-68964-5_7

Billiar, T. R., Cirino, G., Fulton, D., Motterlini, R., Papapetropoulos, A., & Szabo, C. (2019). Nitric oxide synthases (version 2019.4) in the IUPHAR/BPS guide to Pharmacology database. *IUPHAR/BPS Guide to Pharmacology CITE*, *2019*(4), 1–3. https://doi.org/10.2218/gtopdb/F253/2019.4

Brewer, C. L., & Baccei, M. L. (2020). The development of pain circuits and unique effects of neonatal injury. *Journal of Neural Transmission*, *127*(4), 467–479. https://doi.org/10.1007/s00702-019-02059-z

Brown, A. G. (1981). *Organization in the spinal cord: The anatomy and physiology of identified neurones*. Berlin Heidelberg New York: Springer.

Burgoyne, J. R., Madhani, M., Cuello, F., Charles, R. L., Brennan, J. P., Schröder, E., Browning, D. D., & Eaton, P. (2007). Cysteine redox sensor in PKGIa enables oxidant-induced activation. *Science*, *317* (5843), 1393–1397. https://doi.org/10.1126/science.1144318

Cabrera-Pastor, A., Taoro-González, L., Cuñat, A. N., Canet-López, D., Balzano, T., & Felipo, V. (2017). Extracellular cyclic GMP modulates membrane expression of the GluA1 and GluA2 subunits of AMPA receptor in cerebellum: Molecular mechanisms involved. *Scientific Reports*, *7*(1), 1–13. https://doi.org/10.1038/s41598-017-18024-3

Castro, J., Harrington, A. M., Hughes, P. A., Martin, C. M., Ge, P., Shea, C. M., Jin, H., Jacobson, S., Hannig, G., Mann, E., Cohen, M. B., MacDougall, J. E., Lavins, B. J., Kurtz, C. B., Silos-Santiago, I., Johnston, J. M., Currie, M. G., Blackshaw, L. A., & Brierley, S. M. (2013). Linaclotide inhibits colonic nociceptors and relieves abdominal pain via guanylate cyclase-C and extracellular cyclic guanosine 3′,5′-monophosphate. *Gastroenterology*, *145*(6), 1334–1346. http://doi.org/10.1053/j.gastro.2013.08.017

Chang, P., Fabrizi, L., Olhede, S., & Fitzgerald M. (2016). The development of nociceptive network activity in the somatosensory cortex of freely moving rat pups. *Cerebral Cortex*, *26*(12), 4513–4523. http://doi.org/10.1093/cercor/bhw330

Chédotal, A. (2019). Roles of axon guidance molecules in neuronal wiring in the developing spinal cord. *Nature Reviews Neuroscience*, *20*(7), 380–396. https://doi.org/10.1038/s41583-019-0168-7

Cheepala, S., Hulot, J.-S., Morgan, J. A., Sassi, Y., Zhang, W., Naren, A. P., & Schuetz, J. D. (2013). Cyclic nucleotide compartmentalization: Contributions of phosphodiesterases and ATP-binding cassette transporters. *Annual Review of Pharmacology and Toxicology*, *53*, 231–253. https://doi.org/10.1146/annurev-pharmtox-010611-134609

Chen, S.-R., Jin, X.-G., & Pan, H.-L. (2017). Endogenous nitric oxide inhibits spinal NMDA receptor activity and pain hypersensitivity induced by nerve injury. *Neuropharmacology*, *125*, 156–165. https://doi.org/10.1016/j.neuropharm.2017.07.023

Choi, S., Hachisuka, J., Brett, M. A., Magee, A. R., Omori, Y., Iqbal, N.-U.-A., Zhang, D., DeLisle, M. M., Wolfson, R. L., Bai, L., Santiago, C., Gong, S., Goulding, M., Heintz, N., Koerber, H. R., Ross, S. E., & Ginty, D. D. (2020). Parallel ascending spinal pathways for affective touch and pain. *Nature*, *587*(7833), 258–263. https://doi.org/10.1038/s41586-020-2860-1

Chu, Y.-C., Guan, Y., Skinner, J., Raja, S. N., Johns, R. A., & Tao, Y.-X. (2005). Effect of genetic knockout or pharmacologic inhibition of neuronal nitric oxide synthase on complete Freund's adjuvant-induced persistent pain. *Pain*, *119*(1–3), 113–123. https://doi.org/10.1016/j.pain.2005.09.024

Costa, A., Galdino, G., Romero, T., Silva, G., Cortes, S., Santos, R., & Duarte, I. (2014). Ang-(1-7) activates the NO/cGMP and ATP-sensitive K+ channels pathway to induce peripheral antinociception in rats. *Nitric Oxide*, *37*, 11–16. https://doi.org/10.1016/j.niox.2013.12.007

de Carvalho Veloso, C., Rodrigues, V. G., Ferreira, R. C. M., Duarte, L. P., Klein, A., Duarte, I. D., Romero, T. R., & de Castro Perez, A. (2015). Tingenone, a pentacyclic triterpene, induces peripheral antinociception due to NO/cGMP and ATP-sensitive K(+) channels pathway activation in mice. *European Journal of Pharmacology*, *755*, 1–5. https://doi.org/10.1016/j.ejphar.2015.02.038

Ding, J.-D., & Weinberg, R. J. (2006). Localization of soluble guanylyl cyclase in the superficial dorsal horn. *The Journal of Comparative Neurology*, *495*(6), 668–678. https://doi.org/10.1002/cne.20901

Dong, X., & Dong, X. (2018). Peripheral and central mechanisms of itch. *Neuron*, *98*(3), 482–494. https://doi.org/10.1016/j.neuron.2018.03.023

Dumoulin, A., Dagane, A., Dittmar, G., & Rathjen, F. G. (2018). S-palmitoylation is required for the control of growth cone morphology of DRG neurons by CNP-induced cGMP signaling. *Frontiers in Molecular Neuroscience*, *11*, 1–22. https://doi.org/10.3389/fnmol.2018.00345

Emery, E. C., & Ernfors, P. (2020). Dorsal root ganglion neuron types and their functional specialization. In J. N. Wood (Ed.), *[Oxford handbooks]. The Oxford handbook of the neurobiology of pain* (pp. 127–155). New York, NY: Oxford University Press. https://doi.org/10.1093/oxfordhb/9780190860509.013.4

Feil, R., & Kleppisch, T. (2008). NO/cGMP-dependent modulation of synaptic transmission. In T. C. Südhof & K. Starke (Eds.), Handbook of Experimental Pharmacology: Vol. 184. *Pharmacology of neurotransmitter release* (pp. 529–560). Berlin Heidelberg: Springer. https://doi.org/10.1007/978-3-540-74805-2_16

Fitzgerald, M. (2005). The development of nociceptive circuits. *Nature Reviews Neuroscience*, *6*(7), 507–520. https://doi.org/10.1038/nrn1701

Friebe, A., Sandner, P., & Schmidtko, A. (2020). cGMP: A unique 2nd messenger molecule—Recent developments in cGMP research and development. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *393*(2), 287–302. https://doi.org/10.1007/s00210-019-01779-z

Gangadharan, V., Wang, X., & Luo, C. (2017). Cyclic GMP-dependent protein kinase-I localized in nociceptors modulates nociceptive cortical neuronal activity and pain hypersensitivity. *Molecular Pain*, *13*, 1744806917701743. https://doi.org/10.1177/1744806917701743

Gao, Y., Liu, Z., Sun, D.-M., Huang, L., Ma, L.-P., Yin, L.-C., Ma, T., Zhang, Z., Ma, X. L., Peng, L. M., Cheng, H. M., & Ren, W. (2015). Large-area synthesis of high-quality and uniform monolayer WS2 on reusable au foils. *Nature Communications*, *6*, 1–10. https://doi.org/10.1038/ncomms9569

Gbekor, E., Betheil, S., Fawcett, L., Mount, N., & Phillips, S. (2002). Selectivity of sildenafil and other phosphodiesterase type 5 (PDE5) inhibitors against all human phosphodiesterase families. *European Urology Supplements*, *1*(1), 63. https://doi.org/10.1016/S1569-9056(02)80240-8

Gediz, E. İ., Nacitarhan, C., Minareci, E., & Sadan, G. (2015). Antinociceptive effect of vardenafil on carrageenan-induced hyperalgesia in rat: Involvement of nitric oxide/cyclic guanosine monophosphate/calcium channels pathway. *Iranian Journal of Pharmaceutical Research*, *14*(4), 1137–1143.

Guan, Y., Yaster, M., Raja, S. N., & Tao, Y.-X. (2007). Genetic knockout and pharmacologic inhibition of neuronal nitric oxide synthase attenuate nerve injury-induced mechanical hypersensitivity in mice. *Molecular Pain*, *3*(29), 1–10. https://doi.org/10.1186/1744-8069-3-29

Gundersen, R. W., & Barrett, J. N. (1980). Characterization of the turning response of dorsal root neurites toward nerve growth factor. *The Journal of Cell Biology*, *87*(3 Pt 1), 546–554. https://doi.org/10.1083/jcb.87.3.546

Guo, D., Tan, Y., Wang, D., Madhusoodanan, K. S., Zheng, Y., Maack, T., Zhang, J. J., & Huang, X.-Y. (2007). A Rac-cGMP signaling pathway. *Cell*, *128*(2), 341–355. https://doi.org/10.1016/j.cell.2006.11.048

Hanani, M., & Spray, D. C. (2020). Emerging importance of satellite glia in nervous system function and dysfunction. *Nature Reviews Neuroscience*, *21*(9), 485–498. https://doi.org/10.1038/s41583-020-0333-z

Häring, M., Zeisel, A., Hochgerner, H., Rinwa, P., Jakobsson, J. E. T., Lönnerberg, P., La Manno, G., Sharma, N., Borgius, L., Kiehn, O., Lagerström, M. C., Linnarsson, S., & Ernfors, P. (2018). Neuronal atlas of the dorsal horn defines its architecture and links sensory input to transcriptional cell types. *Nature Neuroscience*, *21*(6), 869–880. https://doi.org/10.1038/s41593-018-0141-1

Heine, S., Michalakis, S., Kallenborn-Gerhardt, W., Lu, R., Lim, H.-Y., Weiland, J., Del Turco, D., Deller, T., Tegeder, I., Biel, M., Geisslinger, G., & Schmidtko, A. (2011). CNGA3: A target of spinal nitric oxide/cGMP signaling and modulator of inflammatory pain hypersensitivity. *The Journal of Neuroscience*, *31*(31), 11184–11192. https://doi.org/10.1523/JNEUROSCI.6159-10.2011

Hofmann, F. (2020). The cGMP system: Components and function. *Biological Chemistry*, *401*(4), 447–469. https://doi.org/10.1515/hsz-2019-0386

Huang, J., Gadotti, V. M., Chen, L., Souza, I. A., Huang, S., Wang, D., Ramakrishnan, C., Deisseroth, K., Zhang, Z., & Zamponi, G. W. (2019). A neuronal circuit for activating descending modulation of neuropathic pain. *Nature Neuroscience*, *22*(10), 1659–1668. https://doi.org/10.1038/s41593-019-0481-5

Huang, J., Polgár, E., Solinski, H. J., Mishra, S. K., Tseng, P.-Y., Iwagaki, N., Boyle, K. A., Dickie, A. C., Kriegbaum, M. C., Wildner, H., Zeilhofer, H. U., Watanabe, M., Riddell, J. S., Todd, A. J., & Hoon, M. A. (2018). Circuit dissection of the role of somatostatin in itch and pain. *Nature Neuroscience*, *21*(5), 707–716. https://doi.org/10.1038/s41593-018-0119-z

Hughes, D. I., & Todd, A. J. (2020). Central nervous system targets: Inhibitory interneurons in the spinal cord. *Neurotherapeutics*, *17*(3), 874–885. https://doi.org/10.1007/s13311-020-00936-0

Jacobs, K. M. (2018). Somatosensory system. In J. S. Kreutzer, J. DeLuca, & B. Caplan (Eds.), *Encyclopedia of clinical neuropsychology* (pp. 3218–3223). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-57111-9_359

Jain, N. K., Patil, C. S., Singh, A., & Kulkarni, S. K. (2001). Sildenafil-induced peripheral analgesia and activation of the nitric oxide-cyclic GMP pathway. *Brain Research*, *909*(1–2), 170–178. https://doi.org/10.1016/s0006-8993(01)02673-7

Jin, X. H., Siragy, H. M., & Carey, R. M. (2001). Renal interstitial cGMP mediates natriuresis by direct tubule mechanism. *Hypertension*, *38*(3), 309–316. https://doi.org/10.1161/01.hyp.38.3.309

Kalil, K., & Dent, E. W. (2014). Branch management: Mechanisms of axon branching in the developing vertebrate CNS. *Nature Reviews Neuroscience*, *15*(1), 7–18. https://doi.org/10.1038/nrn3650

Kallenborn-Gerhardt, W., Lu, R., Bothe, A., Thomas, D., Schlaudraff, J., Lorenz, J. E., Lippold, N., Real, C. I., Ferreirós, N., Geisslinger, G., Del Turco, D., & Schmidtko, A. (2014). Phosphodiesterase 2A localized in the spinal cord contributes to inflammatory pain processing. *Anesthesiology*, *121*(2), 372–382. https://doi.org/10.1097/ALN.0000000000000270

Kallenborn-Gerhardt, W., Metzner, K., Lu, R., Petersen, J., Kuth, M. S., Heine, S., Drees, O., Paul, M., Becirovic, E., Kennel, L., Flauaus, C., Gross, T., Wack, G., Hohmann, S. W., Nemirovski, D., Del Turco, D., Biel, M., Geisslinger, G., Michalakis, S., & Schmidtko, A. (2020). Neuropathic and cAMP-induced pain behavior is ameliorated in mice lacking CNGB1. *Neuropharmacology*, *171*, 108087. https://doi.org/10.1016/j.neuropharm.2020.108087

Kallenborn-Gerhardt, W., Schröder, K., Del Turco, D., Lu, R., Kynast, K., Kosowski, J., Niederberger, E., Shah, A. M., Brandes, R. P., Geisslinger, G., & Schmidtko, A. (2012). NADPH oxidase-4 maintains neuropathic pain after peripheral nerve injury. *The Journal of Neuroscience*, *32*(30), 10136–10145. https://doi.org/10.1523/JNEUROSCI.6227-11.2012

Kallenborn-Gerhardt, W., Schröder, K., Geisslinger, G., & Schmidtko, A. (2013). NOXious signaling in pain processing. *Pharmacology & Therapeutics*, *137*(3), 309–317. https://doi.org/10.1016/j.pharmthera.2012.11.001

Kobayashi, T., Nagase, F., Hotta, K., & Oka, K. (2013). Crosstalk between second messengers predicts the motility of the growth cone. *Scientific Reports*, *3*, 1–5. https://doi.org/10.1038/srep03118

Koesling, D., Mergia, E., & Russwurm, M. (2016). Physiological functions of NO-sensitive guanylyl cyclase isoforms. *Current Medicinal Chemistry*, *23*(24), 2653–2665. https://doi.org/10.2174/0929867323666160812145050

Kuhn, M. (2016). Molecular physiology of membrane guanylyl cyclase receptors. *Physiological Reviews*, *96*(2), 751–804. https://doi.org/10.1152/physrev.00022.2015

Liu, X., Wang, Y., Tao, T., Zeng, L., Wang, D., Wen, Y., Li, Y., Zhao, Z., Tao, & A. (2021). GRPR/Extracellular signal–regulated kinase and NPRA/extracellular signal–regulated kinase signaling pathways play a critical role in spinal transmission of chronic itch. *Journal of Investigative Dermatology*, *141*(4), 863–873. http://doi.org/10.1016/j.jid.2020.09.008

Loo, L., Shepherd, A. J., Mickle, A. D., Lorca, R. A., Shutov, L. P., Usachev, Y. M., & Mohapatra, D. P. (2012). The C-type natriuretic peptide induces thermal hyperalgesia through a noncanonical Gβγ-dependent modulation of TRPV1 channel. *The Journal of Neuroscience*, *32*(35), 11942–11955. https://doi.org/10.1523/JNEUROSCI.1330-12.2012

Lorenz, J. E., Kallenborn-Gerhardt, W., Lu, R., Syhr, K. M. J., Eaton, P., Geisslinger, G., & Schmidtko, A. (2014). Oxidant-induced activation of cGMP-dependent protein kinase Iα mediates neuropathic pain after peripheral nerve injury. *Antioxidants & Redox Signaling*, *21*(10), 1504–1515. https://doi.org/10.1089/ars.2013.5585

Lu, C. C., Appler, J. M., Houseman, E. A., & Goodrich, L. V. (2011). Developmental profiling of spiral ganglion neurons reveals insights into auditory circuit assembly. *The Journal of Neuroscience*, *31*(30), 10903–10918. https://doi.org/10.1523/JNEUROSCI.2358-11.2011

Lu, C. C., Cao, X.-J., Wright, S., Ma, L., Oertel, D., & Goodrich, L. V. (2014). Mutation of Npr2 leads to blurred tonotopic organization of central auditory circuits in mice. *PLoS Genetics*, *10*(12), e1004823. https://doi.org/10.1371/journal.pgen.1004823

Luo, C., Gangadharan, V., Bali, K. K., Xie, R.-G., Agarwal, N., Kurejova, M., Tappe-Theodor, A., Tegeder, I., Feil, S., Lewin, G., Polgar, E., Todd, A. J., Schlossmann, J., Hofmann, F., Liu, D. L., Hu, S. J., Feil, R., Kuner, T., & Kuner, R. (2012). Presynaptically localized cyclic GMP-dependent protein kinase 1 is a key determinant of spinal synaptic potentiation and pain hypersensitivity. *PLoS Biology*, *10*(3), e1001283. https://doi.org/10.1371/journal.pbio.1001283

Luo, Z. D., & Cizkova, D. (2000). The role of nitric oxide in nociception. *Current Review of Pain*, *4*(6), 459–466. https://doi.org/10.1007/s11916-000-0070-y

Ma, L., & Tessier-Lavigne, M. (2007). Dual branch-promoting and branch-repelling actions of slit/Robo signaling on peripheral and central branches of developing sensory axons. *The Journal of Neuroscience*, *27*(25), 6843–6851. https://doi.org/10.1523/JNEUROSCI.1479-07.2007

Meller, S. T., & Gebhart, G. F. (1993). Nitric oxide (NO) and nociceptive processing in the spinal cord. *Pain*, *52*(2), 127–136. https://doi.org/10.1016/0304-3959(93)90124-8

Meng, J., Chen, W., & Wang, J. (2020). Interventions in the B-type natriuretic peptide signalling pathway as a means of controlling chronic itch. *British Journal of Pharmacology*, *177*(5), 1025–1040. https://doi.org/10.1111/bph.14952

Meng, J., Moriyama, M., Feld, M., Buddenkotte, J., Buhl, T., Szöllösi, A., Zhang, J., Miller, P., Ghetti, A., Fischer, M., Reeh, P. W., Shan, C., Wang, J., & Steinhoff, M. (2018). New mechanism underlying IL-31–induced atopic dermatitis. *Journal of Allergy and Clinical Immunology*, *141*(5), 1677–1689. http://doi.org/10.1016/j.jaci.2017.12.1002

Michalakis, S., Becirovic, E., & Biel, M. (2018). Retinal cyclic nucleotide-gated channels: From pathophysiology to therapy. *International Journal of Molecular Sciences*, *19*(3), 1–15. https://doi.org/10.3390/ijms19030749

Mishra, S. K., & Hoon, M. A. (2013). The cells and circuitry for itch responses in mice. *Science*, *340*(6135), 968–971. https://doi.org/10.1126/science.1233765

Murray, A. J., Peace, A. G., & Shewan, D. A. (2009). cGMP promotes neurite outgrowth and growth cone turning and improves axon regeneration on spinal cord tissue in combination with cAMP. *Brain Research*, *1294*, 12–21. https://doi.org/10.1016/j.brainres.2009.07.071

Nguyen-Ba-Charvet, K. T., Brose, K., Marillat, V., Sotelo, C., Tessier-Lavigne, M., & Chédotal, A. (2001). Sensory axon response to substrate-bound Slit2 is modulated by laminin and cyclic GMP. *Molecular and Cellular Neurosciences*, *17*(6), 1048–1058. https://doi.org/10.1006/mcne.2001.0994

Nikolaev, V. O., Gambaryan, S., & Lohse, M. J. (2006). Fluorescent sensors for rapid monitoring of intracellular cGMP. *Nature Methods*, *3*(1), 23–25. https://doi.org/10.1038/nmeth816

Nishiyama, M., Hoshino, A., Tsai, L., Henley, J. R., Goshima, Y., Tessier-Lavigne, M., Poo, M. M., & Hong, K. (2003). Cyclic AMP/GMP-dependent modulation of Ca2+ channels sets the polarity of nerve growth-cone turning. *Nature*, *423*(6943), 990–995. https://doi.org/10.1038/nature01751

Nugent, A. A., Kolpak, A. L., & Engle, E. C. (2012). Human disorders of axon guidance. *Current Opinion in Neurobiology*, *22*(5), 837–843. https://doi.org/10.1016/j.conb.2012.02.006

O'Leary, D. D., Bicknese, A. R., de Carlos, J. A., Heffner, C. D., Koester, S. E., Kutka, L. J., & Terashima, T. (1990). Target selection by cortical axons: Alternative mechanisms to establish axonal connections in the developing brain. *Cold Spring Harbor Symposia on Quantitative Biology*, *55*, 453–468. https://doi.org/10.1101/sqb.1990.055.01.045

Ooashi, N., Futatsugi, A., Yoshihara, F., Mikoshiba, K., & Kamiguchi, H. (2005). Cell adhesion molecules regulate Ca2+−mediated steering of growth cones via cyclic AMP and ryanodine receptor type 3. *The Journal of Cell Biology*, *170*(7), 1159–1167. https://doi.org/10.1083/jcb.200503157

Otari, K. V., & Upasani, C. D. (2015). Involvement of NO-cGMP pathway in anti-hyperalgesic effect of PDE5 inhibitor tadalafil in experimental hyperalgesia. *Inflammopharmacology*, *23*(4), 187–194. https://doi.org/10.1007/s10787-015-0240-5

Otto, G. (2019). Novel targets for itch treatment. *Nature Reviews Drug Discovery*, *18*(9), 666–666. http://doi.org/10.1038/d41573-019-00126-4

Ozaki, S., & Snider, W. D. (1997). Initial trajectories of sensory axons toward laminar targets in the developing mouse spinal cord. *The Journal of Comparative Neurology*, *380*(2), 215–229.

Pagani, M., Albisetti, G. W., Sivakumar, N., Wildner, H., Santello, M., Johannssen, H. C., & Zeilhofer, H. U. (2019). How gastrin-releasing peptide opens the spinal gate for itch. *Neuron*, *103*(1), 102–117.e5. https://doi.org/10.1016/j.neuron.2019.04.022

Patil, C. S., Jain, N. K., Singh, V. P., & Kulkarni, S. K. (2004). Cholinergic-NO-cGMP mediation of sildenafil-induced antinociception. *Indian Journal of Experimental Biology*, *42*(4), 361–367.

Peirs, C., & Seal, R. P. (2016). Neural circuits for pain: Recent advances and current views. *Science*, *354*(6312), 578–584. https://doi.org/10.1126/science.aaf8933

Petersen, J., Mergia, E., Kennel, L., Drees, O., Steubing, R. D., Real, C. I., Kallenborn-Gerhardt, W., Lu, R., Friebe, A., Koesling, D., & Schmidtko, A. (2019). Distinct functions of soluble guanylyl cyclase isoforms NO-GC1 and NO-GC2 in inflammatory and neuropathic pain processing. *Pain*, *160*(3), 607–618. https://doi.org/10.1097/j.pain.0000000000001440

Piper, M., van Horck, F., & Holt, C. (2007). The role of cyclic nucleotides in axon guidance. In D. Bagnard (Ed.), Advances in Experimental Medicine and Biology: Vol. 621. *Axon growth and guidance* (pp. 134–143). https://doi.org/10.1007/978-0-387-76715-4_10

Pitake, S., DeBrecht, J., & Mishra S. K. (2017). Brain natriuretic peptide-expressing sensory neurons are not involved in acute, inflammatory, or neuropathic pain. *Molecular Pain*, *13*, 1–7. http://doi.org/10.1177/1744806917736993

Podda, M. V., & Grassi, C. (2014). New perspectives in cyclic nucleotide-mediated functions in the CNS: The emerging role of cyclic nucleotide-gated (CNG) channels. *Pflügers Archiv / European Journal of Physiology*, *466*(7), 1241–1257. https://doi.org/10.1007/s00424-013-1373-2

Potter, L. R. (2011). Guanylyl cyclase structure, function and regulation. *Cellular Signalling*, *23*(12), 1921–1926. https://doi.org/10.1016/j.cellsig.2011.09.001

Potter, L. R., & Hunter, T. (1998). Identification and characterization of the major phosphorylation sites of the B-type natriuretic peptide receptor. *The Journal of Biological Chemistry*, *273*(25), 15533–15539. https://doi.org/10.1074/jbc.273.25.15533

Prysyazhna, O., Rudyk, O., & Eaton, P. (2012). Single atom substitution in mouse protein kinase G eliminates oxidant sensing to cause hypertension. *Nature Medicine*, *18*(2), 286–290. https://doi.org/10.1038/nm.2603

Qin, L., Sankaran, B., Aminzai, S., Casteel, D. E., & Kim, C. (2018). Structural basis for selective inhibition of human PKG Iα by the balanol-like compound N46. *Journal of Biological Chemistry*, *293*(28), 10985–10992. https://doi.org/10.1074/jbc.ra118.002427

Robinson, J. W., Egbert, J. R., Davydova, J., Schmidt, H., Jaffe, L. A., & Potter, L. R. (2017). Dephosphorylation is the mechanism of fibroblast growth factor inhibition of guanylyl cyclase-B. *Cellular Signalling*, *40*, 222–229. https://doi.org/10.1016/j.cellsig.2017.09.021

Ros, O., Zagar, Y., Ribes, S., Baudet, S., Loulier, K., Couvet, S., Ladarre, D., Aghaie, A., Louail, A., Petit, C., Mechulam, Y., Lenkei, Z., & Nicol, X. (2019). SponGee: A genetic tool for subcellular and cell-specific cGMP manipulation. *Cell Reports*, *27*(13), 4003–4012.e6. https://doi.org/10.1016/j.celrep.2019.05.102

Ruscheweyh, R., Goralczyk, A., Wunderbaldinger, G., Schober, A., & Sandkühler, J. (2006). Possible sources and sites of action of the nitric oxide involved in synaptic plasticity at spinal lamina I projection neurons. *Neuroscience*, *141*(2), 977–988. https://doi.org/10.1016/j.neuroscience.2006.04.010

Sachs, D., Cunha, F. Q., & Ferreira, S. H. (2004). Peripheral analgesic blockade of hypernociception: Activation of arginine/NO/cGMP/protein kinase G/ATP-sensitive K+ channel pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(10), 3680–3685. https://doi.org/10.1073/pnas.0308382101

Schmidt, H., Dickey, D. M., Dumoulin, A., Octave, M., Robinson, J. W., Kühn, R., Feil, R., Potter, L. R., & Rathjen, F. G. (2018). Regulation of the natriuretic peptide receptor 2 (Npr2) by phosphorylation of Juxtamembrane serine and threonine residues is essential for bifurcation of sensory axons. *The Journal of Neuroscience*, *38*(45), 9768–9780. https://doi.org/10.1523/JNEUROSCI.0495-18.2018

Schmidt, H., & Fritzsch, B. (2019). Npr2 null mutants show initial overshooting followed by reduction of spiral ganglion axon projections combined with near-normal cochleotopic projection. *Cell and Tissue Research*, *378*(1), 15–32. https://doi.org/10.1007/s00441-019-03050-6

Schmidt, H., Peters, S., Frank, K., Wen, L., Feil, R., & Rathjen, F. G. (2016). Dorsal root ganglion axon bifurcation tolerates increased cyclic GMP levels: the role of phosphodiesterase 2A and scavenger receptor Npr3. *European Journal of Neuroscience*, *44*(12), 2991–3000. http://doi.org/10.1111/ejn.13434

Schmidt, H., & Rathjen, F. G. (2011). DiI-labeling of DRG neurons to study axonal branching in a whole mount preparation of mouse embryonic spinal cord. *Journal of Visualized Experiments*, (58), 1–6. http://doi.org/10.3791/3667

Schmidt, H., Stonkute, A., Jüttner, R., Koesling, D., Friebe, A., & Rathjen, F. G. (2009). C-type natriuretic peptide (CNP) is a bifurcation factor for sensory neurons. *Proceedings of the National Academy of Sciences*, *106*(39), 16847–16852. https://doi.org/10.1073/pnas.0906571106

Schmidt, H., Stonkute, A., Jüttner, R., Schäffer, S., Buttgereit, J., Feil, R., Feil, R., Hofmann, F., & Rathjen, F. G. (2007). The receptor guanylyl cyclase Npr2 is essential for sensory axon bifurcation within the spinal cord. *The Journal of Cell Biology*, *179*(2), 331–340. https://doi.org/10.1083/jcb.200707176

Schmidt, H., Ter-Avetisyan, G., & Rathjen, F. G. (2013). A genetic strategy for the analysis of individual axon morphologies in cGMP signalling mutant mice. In T. Krieg & R. Lukowski (Eds.), Methods in Molecular Biology: Vol. 1020. *Guanylate Cyclase and Cyclic GMP: Methods and Protocols* (pp. 193–204). New York, Heidelberg, Dordrecht, London: Springer. https://doi.org/10.1007/978-1-62703-459-3_12

Schmidt, H., Werner, M., Heppenstall, P., Henning, M., Moré, M., Kühbandner, S., Lewin, G. R., Hofmann, F., Feil, R., & Rathjen, F. (2002). cGMP-mediated signaling via cGKIalpha is required for the guidance and connectivity of sensory axons. *The Journal of Cell Biology*, *159*(3), 489–498. https://doi.org/10.1083/jcb.200207058

Schmidtko, A. (2015). Nitric oxide-mediated pain processing in the spinal cord. In H. G. Schaible (Ed.), Handbook of Experimental Pharmacology: Vol. 227. *Pain control* (pp. 103–117). Berlin Heidelberg: Springer. https://doi.org/10.1007/978-3-662-46450-2_6

Schmidtko, A., Gao, W., Sausbier, M., Rauhmeier, I., Sausbier, U., Niederberger, E., Scholich, K., Huber, A., Neuhuber, W., Allescher, H. D., Hofmann, F., Tegeder, I., Ruth, P., & Geisslinger, G. (2008). Cysteine-rich protein 2, a novel downstream effector of cGMP/cGMP-dependent protein kinase I-mediated persistent inflammatory pain. *The Journal of Neuroscience*, *28*(6), 1320–1330. https://doi.org/10.1523/JNEUROSCI.5037-07.2008

Schmidtko, A., Tegeder, I., & Geisslinger, G. (2009). No NO, no pain? The role of nitric oxide and cGMP in spinal pain processing. *Trends in Neurosciences*, *32*(6), 339–346. https://doi.org/10.1016/j.tins.2009.01.010

Sharma, N., Flaherty, K., Lezgiyeva, K., Wagner, D. E., Klein, A. M., & Ginty, D. D. (2020). The emergence of transcriptional identity in somatosensory neurons. *Nature*, *577*(7790), 392–398. https://doi.org/10.1038/s41586-019-1900-1

Shuhaibar, L. C., Robinson, J. W., Vigone, G., Shuhaibar, N. P., Egbert, J. R., Baena, V., Uliasz, T. F., Kaback, D., Yee, S. P., Feil, R., Fisher, M. C., Dealy, C. N., Potter, L. R., & Jaffe, L. A. (2017). Dephosphorylation of the NPR2 guanylyl cyclase contributes to inhibition of bone growth by fibroblast growth factor. *eLife*, *6*, e31343. https://doi.org/10.7554/eLife.31343

Solinski, H. J., Kriegbaum, M. C., Tseng, P.-Y., Earnest, T. W., Gu, X., Barik, A., Chesler, A. T., & Hoon, M. A. (2019). Nppb neurons are sensors of mast cell-induced itch. *Cell Reports*, *26*(13), 3561–3573.e4. https://doi.org/10.1016/j.celrep.2019.02.089

Song, H. J., Ming, G., He, Z., Lehmann, M., McKerracher, L., Tessier-Lavigne, M., & Poo, M. (1998). Conversion of neuronal growth cone responses from repulsion to attraction by cyclic nucleotides. *Science*, *281*(5382), 1515–1518. https://doi.org/10.1126/science.281.5382.1515

Song, H. J., & Poo, M. M. (1999). Signal transduction underlying growth cone guidance by diffusible factors. *Current Opinion in Neurobiology*, *9*(3), 355–363. https://doi.org/10.1016/s0959-4388(99)80052-x

Spike, R. C., Puskar, Z., Andrew, D., & Todd, A. J. (2003). A quantitative and morphological study of projection neurons in lamina I of the rat lumbar spinal cord. *European Journal of Neuroscience*, *18*(9), 2433–2448. http://doi.org/10.1046/j.1460-9568.2003.02981.x

Spiller, F., Oliveira Formiga, R., Da Fernandes Silva Coimbra, J., Alves-Filho, J. C., Cunha, T. M., & Cunha, F. Q. (2019). Targeting nitric oxide as a key modulator of sepsis, arthritis and pain. *Nitric Oxide*, *89*, 32–40. https://doi.org/10.1016/j.niox.2019.04.011

Sudoh, T., Kangawa, K., Minamino, N., & Matsuo, H. (1988). A new natriuretic peptide in porcine brain. *Nature*, *332*(6159), 78–81. https://doi.org/10.1038/332078a0

Sun, Y.-G., Zhao, Z.-Q., Meng, X.-L., Yin, J., Liu, X.-Y., & Chen, Z.-F. (2009). Cellular basis of itch sensation. *Science*, *325*(5947), 1531–1534. https://doi.org/10.1126/science.1174868

Sung, Y.-J., Sofoluke, N., Nkamany, M., Deng, S., Xie, Y., Greenwood, J., Farid, R., Landry, D. W., & Ambron, R. T. (2017). A novel inhibitor of active protein kinase G attenuates chronic inflammatory and osteoarthritic pain. *Pain*, *158*(5), 822–832. https://doi.org/10.1097/j.pain.0000000000000832

Tegeder, I., Del Turco, D., Schmidtko, A., Sausbier, M., Feil, R., Hofmann, F., Deller, T., Ruth, P., & Geisslinger, G. (2004). Reduced inflammatory hyperalgesia with preservation of acute thermal nociception in mice lacking cGMP-dependent protein kinase I. *Proceedings of the National Academy of Sciences*, *101*(9), 3253–3257. http://doi.org/10.1073/pnas.0304076101

Ter-Avetisyan, G., Dumoulin, A., Herrel, A., Schmidt, H., Strump, J., Afzal, S., & Rathjen, F. G. (2018). Loss of axon bifurcation in mesencephalic trigeminal neurons impairs the maximal biting force in Npr2-deficient mice. *Frontiers in Cellular Neuroscience*, *12*, 153. http://doi.org/10.3389/fncel.2018.00153

Ter-Avetisyan, G., Rathjen, F. G., & Schmidt, H. (2014). Bifurcation of axons from cranial sensory neurons is disabled in the absence of Npr2-induced cGMP signaling. *The Journal of Neuroscience*, *34*(3), 737–747. https://doi.org/10.1523/JNEUROSCI.4183-13.2014

Thippeswamy, T., & Morris, R. (2002). The roles of nitric oxide in dorsal root ganglion neurons. *Annals of the New York Academy of Sciences*, *962*, 103–110. https://doi.org/10.1111/j.1749-6632.2002.tb04060.x

Thunemann, M., Wen, L., Hillenbrand, M., Vachaviolos, A., Feil, S., Ott, T., Han, X., Fukumura, D., Jain, R. K., Russwurm, M., de Wit, C., & Feil, R. (2013). Transgenic mice for cGMP imaging. *Circulation Research*, *113*(4), 365–371. https://doi.org/10.1161/CIRCRESAHA.113.301063

Todd, A. J. (2010). Neuronal circuitry for pain processing in the dorsal horn. *Nature Reviews Neuroscience*, *11*(12), 823–836. https://doi.org/10.1038/nrn2947

Todd, A. J. (2017). Identifying functional populations among the interneurons in laminae I-III of the spinal dorsal horn. *Molecular Pain*, *13*, 1744806917693003. https://doi.org/10.1177/1744806917693003

Togashi, K., von Schimmelmann, M. J., Nishiyama, M., Lim, C.-S., Yoshida, N., Yun, B., Molday, R. S., Goshima, Y., & Hong, K. (2008). Cyclic GMP-gated CNG channels function in Sema3A-induced growth cone repulsion. *Neuron*, *58*(5), 694–707. https://doi.org/10.1016/j.neuron.2008.03.017

Tojima, T., Itofusa, R., & Kamiguchi, H. (2009). The nitric oxide-cGMP pathway controls the directional polarity of growth cone guidance via modulating cytosolic Ca2+ signals. *The Journal of Neuroscience*, *29*(24), 7886–7897. https://doi.org/10.1523/JNEUROSCI.0087-09.2009

Tröster, P., Haseleu, J., Petersen, J., Drees, O., Schmidtko, A., Schwaller, F., Lewin, G. R., Ter-Avetisyan, G., Winter, Y., Peters, S., Feil, S., Feil, R., Rathjen, F. G., & Schmidt, H. (2018). The absence of sensory axon bifurcation affects nociception and termination fields of afferents in the spinal cord. *Frontiers in Molecular Neuroscience*, *11*, 1–18. https://doi.org/10.3389/fnmol.2018.00019

Uchida, H., Matsumura, S., Katano, T., Watanabe, M., Schlossmann, J., & Ito, S. (2018). Two isoforms of cyclic GMP-dependent kinase-I exhibit distinct expression patterns in the adult mouse dorsal root ganglion. *Molecular Pain*, *14*, 1744806918796409. https://doi.org/10.1177/1744806918796409

Usoskin, D., Furlan, A., Islam, S., Abdo, H., Lönnerberg, P., Lou, D., Hjerling-Leffler, J., Haeggström, J., Kharchenko, O., Kharchenko, P. V., Linnarsson, S., & Ernfors, P. (2015). Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nature Neuroscience*, *18*(1), 145–153. https://doi.org/10.1038/nn.3881

Valek, L., Häussler, A., Dröse, S., Eaton, P., Schröder, K., & Tegeder, I. (2017). Redox-guided axonal regrowth requires cyclic GMP dependent protein kinase 1: Implication for neuropathic pain. *Redox Biology*, *11*, 176–191. https://doi.org/10.1016/j.redox.2016.12.004

Walker, S. M., Beggs, S., & Baccei, M. L. (2016). Persistent changes in peripheral and spinal nociceptive processing after early tissue injury. *Experimental Neurology*, *275*(Pt 2), 253–260. https://doi.org/10.1016/j.expneurol.2015.06.020

Wang, F., Ma, S.-B., Tian, Z.-C., Cui, Y.-T., Cong, X.-Y., Wu, W.-B., Wang, F.-D., Li, Z.-Z., Han, W.-J., Wang, T.-Z., Sun, Z.-C., Zhang, F.-L., Xie, R.-G., Wu, S.-X., & Luo, C. (2021). Nociceptor-localized cGMP-dependent protein kinase I is a critical generator for central sensitization and neuropathic pain. *Pain*, *162*(1), 135–151. http://doi.org/10.1097/j.pain.0000000000002013

Wang, J.-N., Zhao, X.-J., Liu, Z.-H., Zhao, X.-L., Sun, T., & Fu, Z.-J. (2017). Selective phosphodiesterase-2A inhibitor alleviates radicular inflammation and mechanical allodynia in non-compressive lumbar disc herniation rats. *European Spine Journal*, *26*(7), 1961–1968. https://doi.org/10.1007/s00586-017-5023-9

Wang, L., Chopp, M., & Zhang, Z. G. (2017). PDE5 inhibitors promote recovery of peripheral neuropathy in diabetic mice. *Neural Regeneration Research*, *12*(2), 218–219. https://doi.org/10.4103/1673-5374.200804

Watanabe, K., Tamamaki, N., Furuta, T., Ackerman, S. L., Ikenaka, K., & Ono, K. (2006). Dorsally derived netrin 1 provides an inhibitory cue and elaborates the 'waiting period' for primary sensory axons in the developing spinal cord. *Development*, *133*(7), 1379–1387. https://doi.org/10.1242/dev.02312

Wedel, B., Harteneck, C., Foerster, J., Friebe, A., Schultz, G., & Koesling, D. (1995). Functional domains of soluble guanylyl cyclase. *The Journal of Biological Chemistry*, *270*(42), 24871–24875. https://doi.org/10.1074/jbc.270.42.24871

Xia, C., Nguyen, M., Garrison, A. K., Zhao, Z., Wang, Z., Sutherland, C., & Ma, L. (2013). CNP/cGMP signaling regulates axon branching and growth by modulating microtubule polymerization. *Developmental Neurobiology*, *73*(9), 673–687. https://doi.org/10.1002/dneu.22078

Yamazaki, M., Chiba, K., Mohri, T., & Hatanaka, H. (2004). Cyclic GMP-dependent neurite outgrowth by genipin and nerve growth factor in PC12h cells. *European Journal of Pharmacology*, *488*(1–3), 35–43. https://doi.org/10.1016/j.ejphar.2004.02.009

Yoder, A. R., Robinson, J. W., Dickey, D. M., Andersland, J., Rose, B. A., Stone, M. D., Griffin, T. J., & Potter, L. R. (2012). A functional screen provides evidence for a conserved, regulatory, juxtamembrane phosphorylation site in guanylyl cyclase a and B. *PLoS ONE*, *7*(5), e36747. https://doi.org/10.1371/journal.pone.0036747

Yoder, A. R., Stone, M. D., Griffin, T. J., & Potter, L. R. (2010). Mass spectrometric identification of phosphorylation sites in guanylyl cyclase A and B. *Biochemistry*, *49*(47), 10137–10145. https://doi.org/10.1021/bi101700e

Zaccolo, M., & Movsesian, M. A. (2007). cAMP and cGMP signaling cross-talk: Role of phosphodiesterases and implications for cardiac pathophysiology. *Circulation Research*, *100*(11), 1569–1578. https://doi.org/10.1161/CIRCRESAHA.106.144501

Zeisel, A., Hochgerner, H., Lönnerberg, P., Johnsson, A., Memic, F., van der Zwan, J., Häring, M., Braun, E., Borm, L. E., La Manno, G., Codeluppi, S., Furlan, A., Lee, K., Skene, N., Harris, K. D., Hjerling-Leffler, J., Arenas, E., Ernfors, P., Marklund, U., & Linnarsson, S. (2018). Molecular Architecture of the Mouse Nervous System. *Cell*, *174*(4), 999–1014.e22. http://doi.org/10.1016/j.cell.2018.06.021

Zhao, J.-G., Cao, C.-H., Liu, C.-Z., Han, B.-J., Zhang, J., Li, Z.-G., Yu, T., Wang, X. H., Zhao, H., & Xu, Z.-H. (2009). Effect of acupuncture treatment on spastic states of stroke patients. *Journal of the Neurological Sciences*, *276*(1–2), 143–147. https://doi.org/10.1016/j.jns.2008.09.018

Zhao, Z., & Ma, L. (2009). Regulation of axonal development by natriuretic peptide hormones. *Proceedings of the National Academy of Sciences*, *106*(42), 18016–18021. https://doi.org/10.1073/pnas.0906880106

Zhao, Z., Wang, Z., Gu, Y., Feil, R., Hofmann, F., & Ma, L. (2009). Regulate axon branching by the cyclic GMP pathway via inhibition of glycogen synthase kinase 3 in dorsal root ganglion sensory neurons. *The Journal of Neuroscience*, *29*(5), 1350–1360. https://doi.org/10.1523/JNEUROSCI.3770-08.2009

Zimmerman, N. P., Brownfield, M. S., DeVente, J., Bass, P., & Oaks, J. A. (2008). cGMP secreted from the tapeworm *Hymenolepis diminuta* is a signal molecule to the host intestine. *The Journal of Parasitology*, *94*(4), 771–779. https://doi.org/10.1645/GE-1418.1

How to cite this article: Schmidt, H., Böttcher, A., Gross, T., & Schmidtko, A. (2022). cGMP signalling in dorsal root ganglia and the spinal cord: Various functions in development and adulthood. *British Journal of Pharmacology*, *179*(11), 2361–2377. https://doi.org/10.1111/bph.15514