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Review Article

VGLUTs in Peripheral Neurons and the Spinal Cord: Time for a Review

Pablo R. Brumovsky

Faculty of Biomedical Sciences, Austral University, Avenida Juan D. Perón 1500, 1629AHJ Pilar, Buenos Aires, Argentina

Correspondence should be addressed to Pablo R. Brumovsky; pablo_brumovsky@yahoo.com

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Vesicular glutamate transporters (VGLUTs) are key molecules for the incorporation of glutamate in synaptic vesicles across the nervous system, and since their discovery in the early 1990s, research on these transporters has been intense and productive. This review will focus on several aspects of VGLUTs research on neurons in the periphery and the spinal cord. Firstly, it will begin with a historical account on the evolution of the morphological analysis of glutamatergic systems and the pivotal role played by the discovery of VGLUTs. Secondly, and in order to provide an appropriate framework, there will be a synthetic description of the neuroanatomy and neurochemistry of peripheral neurons and the spinal cord. This will be followed by a succinct description of the current knowledge on the expression of VGLUTs in peripheral sensory and autonomic neurons and neurons in the spinal cord. Finally, this review will address the modulation of VGLUTs expression after nerve and tissue insult, their physiological relevance in relation to sensation, pain, and neuroprotection, and their potential pharmacological usefulness.

1. How VGLUTs Became the "Gold Standard" for the Identification of Glutamatergic Neurons

Before focusing on the presence and role of vesicular glutamate transporters in neurons in the periphery and the spinal cord, it is important to begin with some historical facts on how it was that glutamatergic neurons were identified in the nervous system. Several decades of research established that glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS) [1] and PNS, including dorsal root ganglion (DRG) and spinal cord neurons [2, 3]. However, the morphological phenotyping of glutamatergic neurons as well as glial cells was not to be a trivial matter.

First in accomplishing a major breakthrough were Storm-Mathisen, Ottersen, and their colleagues who, by means of careful electron microscopy methodologies and meticulous analysis, demonstrated glutamate-like immunoreactivity (Li) in several areas of the mouse, rat, guinea pig, and monkey brain and, importantly, its association to synapses [4–6]. This pioneering work led to the distinction of a "transmitter pool" in glutamatergic terminals, a "metabolic pool" in nonglutamatergic neurons, and a "glial pool" [7–9]. It also prompted the immunohistochemical analysis in sensory neurons, using antibodies against glutamate [10–14]. Subsequent methods to identify glutamatergic neurons were based on the immunohistochemical detection of enzymes like glutaminase, involved in the synthesis of glutamate [15, 16]. This was being reliably done for other neurotransmitters such as catecholamines, serotonin, acetylcholine, and also GABA [17]. More indirect approaches, like the identification of excitatory amino acid transporters (EAATs) located at the cell membrane, both of neurons and astrocytes, and critical for the removal of glutamate released at the synaptic cleft, also emerged [18, 19]. However, since glutamate is a major participant in cell metabolism, even for the synthesis of the inhibitory neurotransmitter GABA [7, 20], and not always the visualization of associated molecules guarantees the glutamatergic nature of neurons, an ideal marker was still sought.

A second breakthrough took place in the mid 1990s, when Ni and collaborators [21] revealed the presence of a brain-specific Na^+-dependent inorganic phosphate transporter in the brain of rat pups. Further research showed that this transporter was specific to synaptic vesicles, acted as a

vesicular glutamate transporter (VGLUT), and hence was termed $VGLUT_1$ [22, 23]. Soon afterwards, $VGLUT_2$ [24–30] and $VGLUT_3$ [31–33] were discovered and characterized. Importantly, transfection of GABAergic neurons with DNA encoding $VGLUT_1$ [23] or $VGLUT_2$ [29] conferred the capacity to release both glutamate and GABA, confirming their role in glutamate loading of synaptic vesicles.

Thus, it was that the discovery of VGLUTs and the development of selective antibodies and *in situ* hybridization probes for their detection became the "gold standard" for the characterization of glutamatergic neurons in the brain and brainstem [34–37], the spinal cord [38–43], the peripheral nervous system (PNS) [30, 34, 36, 38, 44–66], and even glia [67–70]. It should, however, be mentioned that neurons coexpressing $VGLUT_1$ [71] or $VGLUT_3$ [31] and the GABAergic marker glutamate decarboxylase have been identified in developing rat hippocampal granule cells (GC), in adult rat hippocampal GCs under intense ionotropic or trophic factor stimulation [71] and in interneurons in the stratum radiatum of the hippocampus [31].

2. Some Neuroanatomical and Neurochemical Details of Peripheral Neurons and the Spinal Cord

2.1. Sensory and Autonomic Ganglia. Sensory impulses, including pain, originating in the surface of the body (e.g., the skin) or deeper structures (e.g., muscles, joints, and viscera) are transmitted to the spinal cord by way of peripheral nerves. These are contributed by thousands of axons produced by sensory neurons (also called primary afferent neurons) contained in the DRGs and cranial ganglia [3]. The great majority of studies analyzing the characteristics of primary afferent neurons focus on "nonvisceral" DRGs, more specifically the 4th and 5th lumbar (L4-5) DRGs, which typically innervate muscles and skin of the leg and foot by way of the sciatic nerve, both in rodents and humans [72].

In contrast, visceral organs are characterized for their innervation by two different "extrinsic" currents: (1) the spinal current, including the pelvic (PN) and the lumbar splanchnic (LSN) nerves [73, 74] and (2) the cranial current, contributed by the vagus nerve [75]. These two currents originate in "visceral" DRG and cranial ganglia neurons, identified by means of retrograde tracing from their peripheral nerve endings in thoracic, abdominal, and pelvic organs. In particular, the PN and the LSN innervating the colorectum or the urinary bladder in rat [73, 76] and mouse [73, 76, 77] carry axons derived from: (1) peripheral projections of thoracolumbar (TL; T8-L1) and lumbosacral (LS; L6-S1) DRG neurons [73, 78]; (2) postganglionic projections of sympathetic neurons contained in the lumbar sympathetic chain (LSC); and (3) sympathetic and parasympathetic neurons present in the "mixed" major pelvic ganglion (MPG) [79–81]. In addition, and unlike other tissues and organs in the body, the gut is also provided with its own "intrinsic" autonomic innervation. This includes sensory and motor autonomic neurons found in the myenteric and submucosal plexuses, from the esophagus to the anus, and collectively referred to as enteric neurons, creating an intrinsic neuronal network [81, 82].

In normal conditions, rodent nonvisceral DRG neurons express a multiplicity of neurotransmitters and receptors, often in different combinations, and are generally considered glutamatergic [83, 84]. Three main subpopulations of DRG neurons have been characterized, including: (1) large and medium-sized neurons expressing NF-200; (2) small and medium-sized neurons expressing the calcitonin gene-related peptide (CGRP) and termed "peptidergic"; and (3) small and medium-sized "nonpeptidergic" neurons expressing components of the receptor for the glial-derived neurotrophic factor and binding of isolectin B4 (IB4; from the plant *Griffonia simplicifolia* I) to neuronal glycoproteins and glycolipids [83, 84]. However, the "nonpeptidergic" term should only be applied to subpopulations lacking CGRP, since colocalization between IB4 and CGRP has been shown in rats [85] and mouse [86]. Interestingly, and highlighting the neurochemical complexity of DRG neurons, subpopulations of nonvisceral DRG neurons, lacking both neuropeptides and/or IB4 while expressing the noradrenergic marker tyrosine hydroxylase (TH) [87] or the neuropeptide tyrosine receptor type 2 (Y2R) [88], have also been described.

Several molecules involved in pain sensing (nociception) are expressed by nonvisceral DRG neurons, including the already mentioned CGRP, the transient receptor potential cation channel, subfamily V, member 1 (TRPV1) [89], the P2X purinoceptor 3 (P_2X_3) [90], or the sodium channel NaV1.8 [91]. Finally, other transmitter candidates in DRG neurons are the nucleotide adenosine-triphosphate (ATP) [92, 93], the "gaseous" transmitter nitric oxide [93, 94], and neurotrophic factors such as the glial- and the brain-derived neurotrophic factors [83].

Visceral DRG neurons, like nonvisceral ones, are classically described as being glutamatergic [14] and normally coexpress a variety of molecules, including neuropeptides [95]. Thus, CGRP is synthesized by rat [76, 96–98] and mouse [76, 77] visceral sensory neurons. $TRPV_1$, P_2X_3, IB4 [73], and even TH [99] are also expressed by visceral sensory neurons.

Autonomic neurons are morphologically characterized as sympathetic or parasympathetic, based on their expression of noradrenaline (using TH or the norepinephrine transporter type 1 for identification) or acetylcholine (using choline acetyltransferase or the vesicular acetylcholine transporter (VAChT) for identification), respectively. However, it should be noted that some sympathetic neurons have also been shown to coexpress acetylcholine and neuropeptides [80, 100–102]. Finally, different subpopulations of enteric neurons in the myenteric and submucosal plexuses typically express markers such as TH, VAChT, nitric oxide synthase, and several neuropeptides [103].

2.2. Spinal Cord. Peripheral stimuli processed by primary afferent neurons are transferred to the spinal cord by way of their central axons (dorsal roots). In this manner, they penetrate the gray matter, which is divided into 9 laminae, from dorsal to ventral, and an area around the central canal (area X), as originally characterized through analysis of the

morphology and arrangement of Nissl-stained cell bodies in transverse sections of the cat spinal cord [104]. Neurons in each laminae are arranged in a complex but ordered manner, based on morphology, neurochemical composition, and specific sensory modalities [105–107]. Thus, lamina I, the most superficial of all, receives cutaneous Aδ- and C-fibers [108–111] and muscle and articular afferents [112, 113], as well as visceral afferents [114–116]. Laminae II, subdivided into outer (IIo) with densely packed cells and inner (IIi) [117], receives predominantly unmyelinated C-fiber afferents [110, 118–120]. In addition, Aδ-fibers terminate mostly in lamina IIo [121], and some cutaneous mechanoreceptive Aβ-fibers reach lamina IIi via lamina III [120, 122–125]. Aα/β-fibers are the main afferent projections to laminae III–VI [110, 112, 123–129]. However, a subpopulation of fine C-fibers also penetrates deeply in the dorsal horn, into lamina III [98, 110, 112, 118, 130–132]. Finally, the area X receives a considerable input of visceral afferents [98, 115, 132–134], although terminals contributed by somatic afferents are also present [129, 135].

3. Expression of VGLUTs in Peripheral Visceral and Nonvisceral Glutamatergic DRG and Cranial Sensory Neurons

3.1. Somatic Expression of VGLUTs. The expression of $VGLUT_1$ mRNA in large and some medium-sized L4-5 DRG neurons was first shown (although not quantified) in the rat [48, 51]. Subsequent analysis in the mouse [53, 54] revealed presence of $VGLUT_1$-IR neurons in 12% to 37% of nonvisceral DRG neurons (Figure 1, Table 1), while its transcript has been detected in ~45% of L4-5 DRG neurons of large and medium size [136]. Immunohistochemical signal for $VGLUT_1$ is also observed in large and medium-sized visceral DRG neurons projecting to the mouse colorectum (12% [61]) and urinary bladder (32% [62]) (Figure 1, Table 1). Variations between the reported proportions of $VGLUT_1$-expressing DRG neurons may depend on: (1) differences in protein and gene regulation between DRG levels (TL versus LS versus L4-5); (2) the use of different $VGLUT_1$ antibodies/probes, immunohistochemical and *in situ* hybridization techniques or even mouse strains; and (3) especially when it comes to transcript versus protein mismatches, differential regulatory mechanisms.

In contrast to $VGLUT_1$, $VGLUT_2$ has been found in a large proportion of DRG neurons, spanning different cell soma sizes (Figure 1, Table 1), as initially shown in immunohistochemical studies in DRGs targeting the rat ileum [44] or *in situ* hybridization analysis of lumbar nonvisceral DRGs [51]. In the mouse, from ~65% [54] and up to 90% [137] $VGLUT_2$-IR NPs are present in L4-5 DRGs. Even higher percentages of $VGLUT_2$-IR NPs have been described in visceral DRG neurons innervating the colorectum [61] or the urinary bladder [62]. Confirming the abundance of $VGLUT_2$ in DRG neurons, up to 70% [136] or 82% [137] of mouse L4-5 DRG neurons have been shown to express $VGLUT_2$ mRNA.

Identifying $VGLUT_3$ in peripheral sensory neurons has been more difficult than for the other VGLUTs, mainly due to the unavailability of antibodies that reliably labelled DRG neurons (in fact, this is still the case today). However, using transgenic mice expressing the enhanced green fluorescent protein (EGFP) under the control of $VGLUT_3$ regulatory sequences, Seal et al. [66] showed that around 10-11% of nonvisceral lumbar DRG neurons express $VGLUT_3$ [66]. This has been recently confirmed by *in situ* hybridization, with detection of $VGLUT_3$ mRNA in ~17% of nonvisceral DRG NPs [65, 136], and through the identification of ~19% of transgenic adult mouse nonvisceral DRG NPs expressing the reporter gene Tomato, under the control of the $VGLUT_3$ promoter [65] (Figure 1, Table 1). $VGLUT_3$ is also present in subpopulations of visceral sensory neurons innervating both the colorectum (~10%, Figure 1, Table 1) or the urinary bladder (~18% [62]). $VGLUT_3$ appears mostly expressed in small and medium-sized adult DRG NPs [62, 65, 66, 136]. However, a transient versus persistent expression of $VGLUT_3$ has been proposed, where the transporter is found in large and medium-sized myelinated nonvisceral DRG neurons only during prenatal stages, with the small neuron population remaining $VGLUT_3$-expressing during the adult life [65].

In DRGs projecting to visceral organs, an additional peculiarity is observed. Thus, TL and LS DRGs innervating the mouse colorectum [61] or the urinary bladder [62] contain different proportions of neurons expressing $VGLUT_1$ or $VGLUT_3$ (see Table 2), whilst $VGLUT_2$ is equally abundant at both DRG levels [62]. Similar variations were also observed for other markers. For instance, the transient receptor potential cation channel, subfamily A, member 1 ($TRPA_1$) mRNA is abundant in mouse TL bladder neurons but scarce in LS bladder neurons [138]. On the contrary, TH protein is expressed in threefold (colorectum) and fivefold (urinary bladder) greater proportions in mouse LS DRGs than in TL DRGs [62]. Interestingly, it has been postulated that differences in neurochemical expression between DRG levels could have an impact in the electrophysiological properties of TL and LS visceral afferent neurons [139, 140], as shown in the mouse colorectum, where a higher expression of $TRPV_1$ in TL than in LS DRGs corresponds with a more robust response to applied capsaicin in colorectal TL nerve terminals [140].

The frequent association of $VGLUT_2$-Li to the plasma membrane in nonvisceral [54] and visceral [61, 62] DRG neurons (Figure 1) has raised the question of if somatic glutamatergic release was possible in these neurons [54]. Accumulating evidence suggests that messenger molecules in general are released from the somatic compartment of the neuron [146], including DRG neurons [147–150]. Thus, neuropeptides [147, 148], ATP [149], and even genetically expressed macromolecular tracers in DRG neurons [150] have been shown to undergo somatic release. Interestingly, recent studies in *Xenopus* oocytes transfected with $VGLUT_2$ suggest that this transporter can be found in two different states: (1) serving the traditional role of packaging glutamate into synaptic vesicles for Ca^{2+}-dependent exocytosis, and (2) participating in Ca^{2+}-independent leakage when present in the plasma membrane [151]. Moreover, this dual role appears to be also true for $VGLUT_1$ and $VGLUT_3$ [151]. Whether such states are also a feature in sensory neurons, and VGLUTs

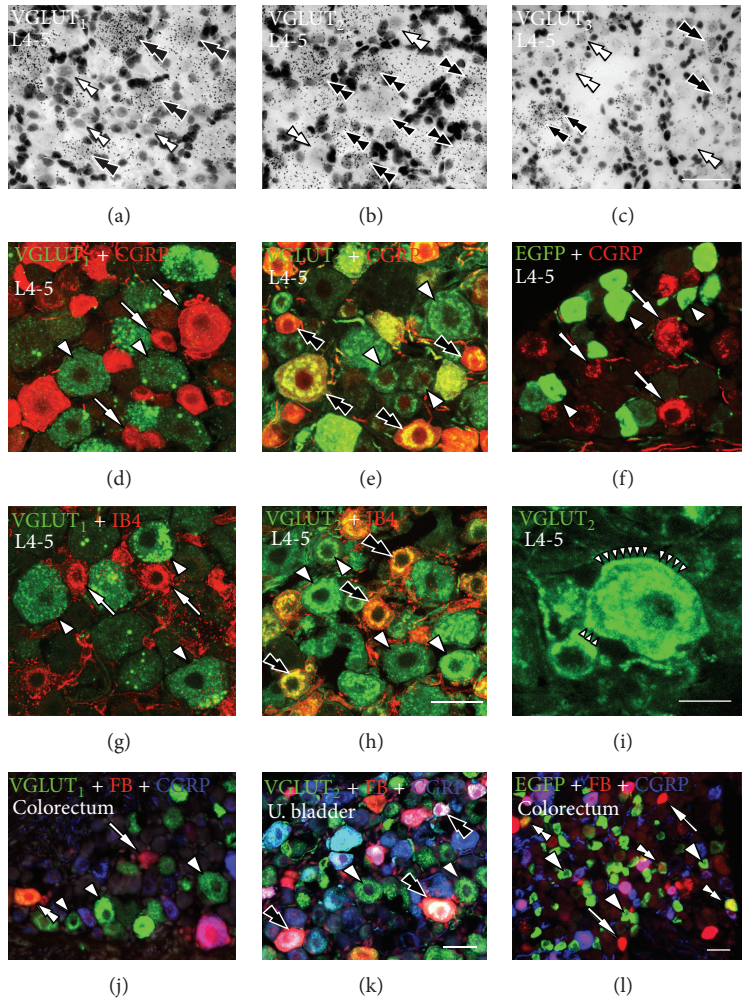


Figure 1: *Broad VGLUT expression in mouse visceral and nonvisceral DRG neurons*. Bright-field ((a)–(c)) and immunofluorescence ((d)–(l)) photomicrographs of DRG sections incubated with $VGLUT_1$, $VGLUT_2$, or $VGLUT_3$ antisense riboprobes ((a), (b), (c), resp.) or $VGLUT_1$ ((d), (g), (j)), $VGLUT_2$ ((e), (h), (i), (k)), or EGFP ((f), (l)) antibodies (EGFP, used as a reporter of $VGLUT_3$ expressing neurons). Colocalizations with CGRP ((d)–(f), (j)–(l)) or IB4 ((g), (h)) are also shown. Colorectum ((j), (l)) or urinary bladder (k) DRG neurons are exposed by presence of the retrograde tracer FB ((j), (k), (l)). ((a)–(c)) VGLUT transcripts are detected in nonvisceral DRGs, $VGLUT_1$ mostly in large and medium-sized NPs (black double arrowheads in (a)), $VGLUT_2$ in many NPs spanning different cell body sizes (black double arrowheads in (b)), and $VGLUT_3$ in a discrete number of usually small and some medium-sized NPs (black double arrowheads in (c)). Many NPs lacking VGLUTs are also detected (white double arrowheads in (a)–(c)). ((d)–(f)) The distribution of $VGLUT_1$- (arrowheads in (d)) $VGLUT_2$- (arrowheads and black double arrowheads in (e)), and EGFP-IR (arrowheads in (f)) nonvisceral DRG NPs correlates with the transcript counterparts shown in (a)–(c). While most CGRP-expressing nonvisceral DRG NPs coexpress with $VGLUT_2$ (black double arrowheads in (e)), $VGLUT_1$-, $VGLUT_3$- (arrowheads in (d), (f)), and CGRP-IR (arrows in (d), (f)) NPs virtually always appear as independent subpopulations. Many $VGLUT_2$-IR DRG NPs lacking CGRP are also detected (arrowheads in (e)). ((g), (h)) IB4 is virtually always present in $VGLUT_2$-IR nonvisceral DRG NPs (black double arrowheads in (h)), while many other $VGLUT_2$-only NPs are also observed (arrowheads in (h)). In contrast, $VGLUT_1$- (arrowheads in (g)) and IB4-expressing (arrows in (g)) DRG NPs always belong to different subpopulations. (i) $VGLUT_2$-membrane staining is detected in a number of nonvisceral DRG NPs (small arrowheads). ((j)–(l)) $VGLUT_1$- (arrowheads and with double arrowhead in (j)), $VGLUT_2$- (arrowheads and black double arrowheads in (k)), and EGFP-IR (arrowheads and white double arrowheads in (l)) are detected in visceral DRG NPs, with a similar cell soma distribution as observed for nonvisceral DRGs. CGRP-IR visceral DRG NPs are virtually always $VGLUT_2$-IR (black double arrowheads in (k)), while NPs expressing the peptide (arrows in (j), (l)) and $VGLUT_1$ (arrowheads and double arrowheads in (j)) or EGFP-IR NPs (arrowheads and double arrowheads in (l)) belong to different subpopulations. Scale bars: 50 μm ((c) = (a), (b); (h) = (d)–(g); (k) = (j); (l)); 20 μm (i). *Data in figures (f) and (l) is previously unpublished, and the tissue has been processed according to the immunohistochemical procedures described in [54, 60–62].*

Table 1: Percentage of DRG neurons (visceral or nonvisceral) expressing VGLUTs (protein and mRNA).

VGLUT	Species	Percent nonvisceral afferents	Percent visceral afferents (target organs)
$VGLUT_1$ protein	*Mouse*	~37% [53]; ~12% [54]	~12% (Colorectum [61]) ~32% (U. Bladder [62])
	Rat	Detected, not quantified [48]	NE (Colorectum, U. Bladder)
$VGLUT_1$ mRNA	*Mouse*	~40% [136]	NE (Colorectum, U. Bladder)
	Rat	Detected, not quantified [48]	NE (Colorectum, U. Bladder)
$VGLUT_2$ protein	*Mouse*	~65% [54]	~97% (Colorectum [61]) ~94% (U. Bladder [62])
	Rat	NE	Detected, not quantified (Stomach and Ileum [44]) NE (Colorectum, U. Bladder)
$VGLUT_2$ mRNA	*Mouse*	~70% [136]	NE (Colorectum, U. Bladder)
	Rat	NE	NE (Colorectum, U. bladder)
$VGLUT_3$ Protein	*Mouse*	~10% [66]; ~19% [65]	~10% (Colorectum*) ~18% (U. Bladder [62])
	Rat	NE	NE (Colorectum, U. bladder)
$VGLUT_3$ mRNA	*Mouse*	~17% [136]; ~16% [65]	NE (Colorectum, U. Bladder)
	Rat	NE	NE (Colorectum, U. Bladder)

NE: not evaluated; *unpublished data; tissue has been processed and NPs quantified according to references [54, 60–62].

Table 2: Percentage of colorectal or urinary bladder DRG neurons expressing VGLUTs protein.

Target organ	DRG level	$VGLUT_1$	$VGLUT_2$	$VGLUT_3$
Colorectum	Thoracolumbar	~15% [61]	~98% [61]	~17%*
	Lumbosacral	~8% [61]	~97% [61]	~4%*
Urinary bladder	Thoracolumbar	~39% [62]	~94% [62]	~28% [62]
	Lumbosacral	~26% [62]	~94% [62]	~8% [62]

*Unpublished data; tissue has been processed and NPs quantified according to references [54, 60–62].

facilitated any form of somatic glutamatergic release remains to be established.

Finally, cranial sensory neurons are richly provided with VGLUTs, and their expression varies between target organs (Table 3).

3.2. Neurochemical Phenotype of VGLUT-Expressing DRG Neurons. As described in Section 2.1, DRG neurons can be divided into peptidergic and nonpeptidergic. $VGLUT_1$-IR DRG neurons, either nonvisceral [48, 53, 54] or visceral [61, 62] appear to be nonpeptidergic and lacking IB4 (Figure 1, Table 4) or TH (Figure 2), as shown in rat [48] and mouse [53, 54, 61, 62]. Li et al. [46] and Alvarez et al. [50], however, have reported that $VGLUT_1$ may be found in neuropeptide containing afferents terminating in the laminae I and II of the rat dorsal horn. This suggests that some peptidergic DRG neurons in the rat may synthesize low levels of $VGLUT_1$, only detected in primary afferent terminals in the spinal cord but not in the larger cell bodies, where the immunohistochemical signal may be "diluted".

Sharing some similarities with $VGLUT_1$, $VGLUT_3$ is expressed in nonvisceral DRG neurons lacking CGRP, only in around 7% of those binding IB4 [66] (Figure 1, Table 4), but in most cases coexpressing with TH (Figure 2), the latter being typically detected in nonpeptidergic, nonvisceral DRG neurons [87]. Accordingly, in visceral DRG neurons targeting either the colorectum (Figure 1) or the urinary bladder [62], $VGLUT_3$ has only been detected in the nonpeptidergic subpopulation (Table 4).

In contrast to $VGLUT_1$ or $VGLUT_3$, a considerable number of $VGLUT_2$-IR DRG neurons coexpress CGRP or IB4 in nonvisceral [54] as well as visceral [61, 62] mouse DRGs (Figure 1, Table 4). Conversely, virtually all DRG neurons expressing CGRP or binding IB4 synthesize $VGLUT_2$. This is in agreement with the considerable colocalization of $VGLUT_2$ and IB4 previously described in primary afferents in the rat dorsal horn [45, 46, 49, 50] and mouse DRG neurons [53] and the previously shown colocalization of glutaminase with CGRP [152] and of glutamate-Li with CGRP or substance P in rat [11, 14, 153]. Accordingly, colorectal—[61] and urinary bladder-projecting [62] DRG neurons often coexpress $VGLUT_2$ and CGRP (Figure 1, Table 4). Finally, virtually all $VGLUT_2$-expressing nonvisceral DRG neurons in the mouse express TH (Figure 2), and in rat [154] and mouse [155], colocalization with TRPV1 has also been reported.

3.3. VGLUTs Colocalization in Cranial and DRG Neurons. It is now clear that at least some neurons in the nervous system express more than only one type of VGLUT. This was not the understanding when VGLUTs were first described. Thus, initial observations in the adult mammalian CNS showed a complementary distribution for $VGLUT_1$ and $VGLUT_2$ [26, 28, 30, 156, 156, 157]. Moreover, this complementarity seemed to extend to the synaptic level, where both VGLUTs appeared

Table 3: Presence of VGLUTs protein in cranial sensory neurons and their projections (upper part, VGLUTs presence in retrogradely traced cranial sensory neurons; lower part, immunohistochemical detection of VGLUTs in nerve terminals of cranial sensory neurons).

Organ/species	Cranial ganglia	$VGLUT_1$	$VGLUT_2$	$VGLUT_3$
Rat stomach [44, 141]	Nodose	+	+	NE
Rat aortic depressor nerve [141, 142]	Nodose	Not detectable	+	+
Guinea pig trachea [58]	Nodose	+	+	NE
Mouse tongue [143]	Geniculate	+	+	NE
Rat cornea [45]	Trigeminal	+	+	NE
Organ/species	Nerve/terminals	$VGLUT_1$	$VGLUT_2$	$VGLUT_3$
Rat pleura [56]	Laminar endings	+	+	NE
Rat heart [141]	Cardiac vagal afferents	+	Not detectable	Not detectable
Guinea pig trachea [58]	Cough mechanoreceptors	+	+	NE
Mouse tongue [143]	Taste Buds	+	+	NE
Rat masseter muscle [144]	Mesencephalic projections	+	NE	NE
Rat cornea [59]	Trigeminal	+	+	NE
Human teeth [63]	Trigeminal	+	+	NE
Rat lung [145]	Pulmonary neuroepithelial bodies	NE	+	NE

+: present; NE: not evaluated.

Table 4: Percentage of DRG neurons (visceral or nonvisceral) expressing VGLUTs and CGRP, IB4, or other VGLUTs.

Colocalization	Species	Percent nonvisceral afferents	Percent visceral afferents (target organ)
$VGLUT_1$ also CGRP	*Mouse*	Not detectable [53, 54]	Not detectable (Colorectum [61], U. Bladder [62])
	Rat	Not detectable [48]	NE (Colorectum, U. bladder)
$VGLUT_1$ also IB4	*Mouse*	Not detectable [53, 54]	NE (Colorectum, U. Bladder)
	Rat	NE	NE (Colorectum, U. Bladder)
$VGLUT_2$ also CGRP	*Mouse*	~31% [54]	~81% (Colorectum [61])
	Rat	NE	~53% (U. Bladder [62])
$VGLUT_2$ also IB4	*Mouse*	~42% [54]	NE (Colorectum, U. Bladder) NE (Colorectum, U. Bladder)
	Rat	NE	NE (Colorectum, U. Bladder)
$VGLUT_3$ also CGRP	*Mouse*	Not detectable [66]	Not detectable (Colorectum*, U. Bladder [62])
	Rat	NE	NE (Colorectum, U. Bladder)
$VGLUT_3$ also IB4	*Mouse*	~7% [66]	NE (Colorectum, U. Bladder)
	Rat	NE	NE (Colorectum, U. Bladder)
$VGLUT_2$ also $VGLUT_1$	*Mouse*	~14% [54]	Highly likely (Colorectum [61], U. Bladder [62])
$VGLUT_2$ also $VGLUT_3$	*Mouse*	~100%*	Highly likely (Colorectum [61], U. Bladder [62])
$VGLUT_1$ also $VGLUT_3$	*Mouse*	Not detectable*	NE (Colorectum, U. Bladder)
$VGLUT_2$ also $VGLUT_1$ $VGLUT_2$ also $VGLUT_3$ $VGLUT_1$ also $VGLUT_3$	*Rat*	NE	NE (Colorectum, U. Bladder)

NE: not evaluated; *unpublished data; tissue has been processed and NPs quantified according to references [54, 60–62].

segregated in physiologically different synapses in the CNS [26, 30, 158, 159]. However, the finding of glutamatergic terminals in the rat hippocampus, containing both $VGLUT_1$ and $VGLUT_2$, suggested the possibility of a supplementary distribution [157].

Such a possibility is in fact also a feature in the periphery. Thus, in rat trigeminal ganglia [45] and nonvisceral DRGs [51], coexpression of $VGLUT_1$ and $VGLUT_2$ protein or mRNA has been reported in a subpopulation of neurons, respectively. This was confirmed in mouse (Table 4), were a moderate coexpression of $VGLUT_1$ and $VGLUT_2$ was shown in some medium-sized and large nonvisceral DRG neurons [54]. Coexpression of $VGLUT_3$ and $VGLUT_2$ (but not $VGLUT_1$) is also detected in nonvisceral DRGs (Figure 2, Table 4). Finally, the overwhelming presence of $VGLUT_2$ in virtually all colorectal [61] and urinary bladder [62]

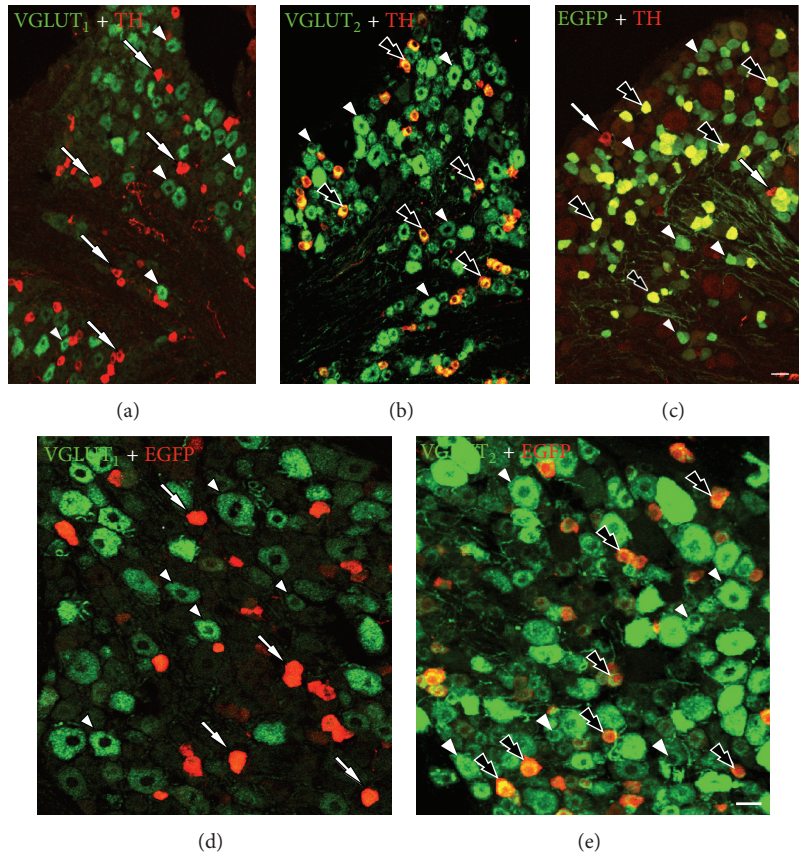


Figure 2: *Frequent coexpression of $VGLUT_2$ or EGFP ($VGLUT_3$) with TH or $VGLUT_2$ and EGFP in mouse nonvisceral DRG neurons.* Immunofluorescence photomicrographs of nonvisceral DRG sections incubated with $VGLUT_1$ ((a), (d)), $VGLUT_2$ ((b), (e)), or EGFP antibodies ((c), (d), (e)). ((a)–(c)) While virtually no $VGLUT_1$-IR (arrowheads in (a)) DRG NPs coexpress with TH (arrows in (a)), most $VGLUT_2$- or EGFP-IR NPs present with TH-Li (black double arrowheads in (b), (c)). Abundant $VGLUT_2$- (arrowheads in (b)), EGFP- (arrowheads in (c)), and some TH-only (arrows in (c)) expressing NPs are also detected. ((d), (e)) Lack of coexpression is observed between $VGLUT_1$ (arrowheads in (d)) and EGFP (arrows in (d)). In contrast, EGFP is virtually always coexpressed with $VGLUT_2$ in DRG NPs (double black arrowheads in (e)). Many other $VGLUT_2$-only expressing DRG NPs are also detected (arrowheads in (e)). Scale bars: 30 μm ((c) = (a), (b); (e) = (d)). *Data in figures (a)–(e) is previously unpublished, and the tissue has been processed according to the immunohistochemical procedures described in [54, 60–62].*

DRG neurons, indirectly implies a considerable degree of colocalization with $VGLUT_1$ or $VGLUT_3$.

How coexpression of two VGLUTs in the same neuron influences its role in neurotransmission is not yet known. It has, however, been suggested that VGLUT expression may be associated with different patterns of neurotransmitter release. Thus, $VGLUT_1$ is normally expressed in CNS neurons with low probability release (climbing fibers in the cerebellum), whereas $VGLUT_2$ would be associated to those with high probability (parallel fibers in the cerebellum) [160]. Whether the type of VGLUT expressed in a single DRG neuron or the coexpression of more than one VGLUT have an impact on release probability remains to be established.

3.4. VGLUTs in the Peripheral Projections of Cranial and DRG Neurons. VGLUTs in peripheral nerves were first identified in rat, mouse [161, 162], and guinea pig [163] esophageal intraganglionic laminar endings (IGLEs), dependent on vagal afferents, and shown to contain $VGLUT_2$. $VGLUT_1$ was also found in esophageal IGLEs in the guinea pig [163] and rat [164] but not the mouse [165]. Subsequent studies revealed presence of VGLUTs in peripheral nerves in many other locations in the upper body (Table 2).

VGLUTs are also present in axonal terminations of abdominopelvic organs in the guinea pig [166] and mouse [61, 62, 167], where $VGLUT_2$ appears to be the main player. Thus, $VGLUT_2$-Li is found in abundant varicosities around $VGLUT_2$-negative mouse colorectal myenteric plexus neurons (Figure 3) [61], in agreement with observations in the guinea pig [166] and mouse rectum [167], and supporting data on guinea pig small intestine showing glutamate-Li with a similar distribution [168]. $VGLUT_2$ is also detected in an overwhelming number of nerve endings terminating in the urinary bladder (Figure 3) [62]. Unlike $VGLUT_2$, $VGLUT_1$ is only found in very few nerve fibers in the mouse colorectum [61], and in a small number of fibers in the urinary bladder, mostly within the muscular layers of this organ (Figure 3) [62]. Discrete $VGLUT_1$ expression has also been reported

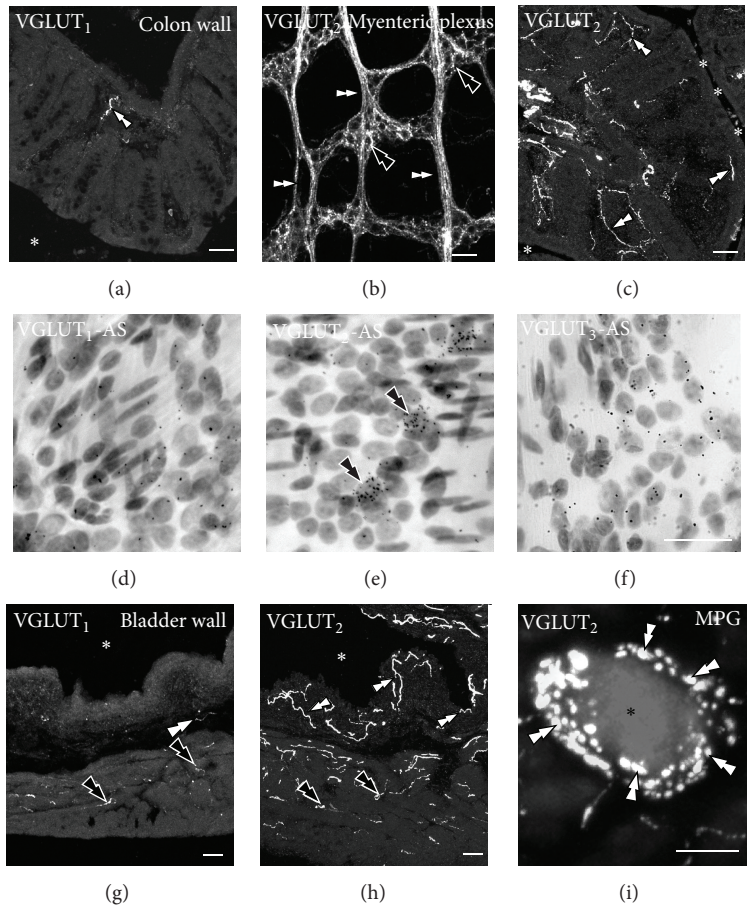


Figure 3: *Distribution of VGLUT-containing peripheral nerve fibers in mouse visceral organs and the MPG*. Immunofluorescence ((a)–(c), (g)–(i)) and bright-field ((d)–(f)) photomicrographs of sections of the colorectum ((a)–(c), (d)–(f)) the urinary bladder ((g), (h)) and the MPG (i), incubated with $VGLUT_1$ ((a), (h)) or $VGLUT_2$ ((b), (c), (h), (i)) antibodies or $VGLUT_1$, $VGLUT_2$ or $VGLUT_3$ antisense riboprobes ((d), (e), (f), resp.). In (i), a colorectal MPG NP is exposed by its content of fast blue (asterisk). In ((a), (c), (g) and (h)), the position of the lumen is indicated by a white asterisk. ((a)–(c)) Isolated $VGLUT_1$-IR nerve fibers are detected in the mucosal layer of the colorectum (double arrowhead in (a)), in contrast to the abundance of $VGLUT_2$-IR nerve fibers in the myenteric plexus (black double arrowheads in (b)), the interconnecting nerve fibers (white double arrowheads in (b)), and the mucosal layer (white double arrowheads in (c)). ((d)–(f)) Only a number of $VGLUT_2$ mRNA-expressing myenteric plexus NPs are detected in the mouse colorectum (black double arrowheads in (e)), in contrast to the absence of $VGLUT_1$- or $VGLUT_3$ mRNA-expressing NPs in such ganglia ((e), (f), resp.). ((g), (h)) While a small number of $VGLUT_1$-IR nerve fibers are detected in the urinary bladder wall, especially in the muscular layer (black double arrowheads in (g)) and some in the submucosa (white double arrowhead in (g)), $VGLUT_2$-IR nerve fibers are abundant and present throughout the whole thickness of the organ, including the muscular (black double arrowheads in (h)) and submucosal and mucosal layers (white double arrowheads in (h)). (i) A colorectal MPG NP is surrounded by a dense $VGLUT_2$-IR basket (white double arrowheads). Scale bars: 50 μm ((a)–(c), (g), (h)); 25 μm ((d)–(f)); 10 μm (i). *Figures (d) and (e) are reproduced in part, and with permission, from reference [61]. Data in figure (f) is previously unpublished, and the tissue has been processed according to the in situ hybridization procedures described in [60, 61].*

in the Pacinian corpuscle and associated neurites in the cat mesentery [55]. The disparate representation of $VGLUT_1$ and $VGLUT_2$ in peripheral nerves terminating in visceral organs is supported by Olsson et al. [166], showing that ~3% of anterogradely traced guinea pig rectal nerve varicosities terminating in the myenteric plexus contain $VGLUT_1$, whereas ~11% exhibit $VGLUT_2$-Li.

In the skin, the immunohistochemical presence of $VGLUT_1$ and $VGLUT_2$ has been studied in mouse [54] and rat [52, 169]. Thus, $VGLUT_1$ is discretely expressed in dermal and epidermal nerves of the glabrous (hairless) skin, in the piloneural complex in hairy skin of mouse (Figure 4) [54], and in rat primary afferent endings in the muscle spindles in the triceps surae muscle [52]. Conversely, $VGLUT_2$ is detected not only in piloneural complexes in hairy skin, but also in numerous nerve endings terminating in the glabrous hindpaw skin (Figure 4), both in deep dermal bundles as well as in close relation to the epidermis, often contacting $VGLUT_2$-IR Merkel cells [54]. The presence of both $VGLUT_1$- and/or $VGLUT_2$-IR fibers in the piloneural complex suggests their origin in glutamatergic DRG neurons producing myelinated D-fibers [170].

Peripheral nerve endings containing $VGLUT_3$ have been more difficult to analyze than those expressing $VGLUT_1$

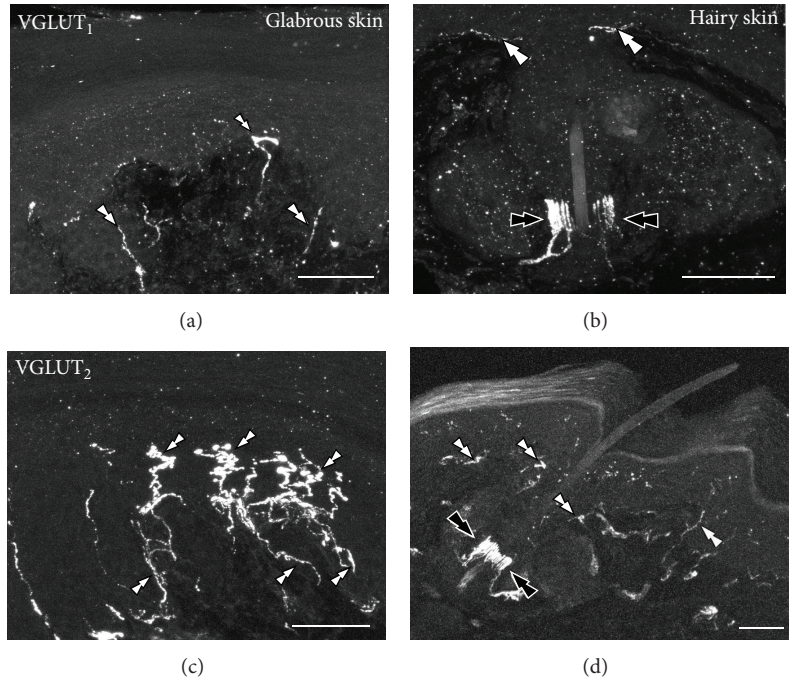


Figure 4: *Distribution of VGLUT-containing peripheral nerve fibers in the mouse skin.* Immunofluorescence photomicrographs of sections of the glabrous ((a), (c)) and hairy skin ((b), (d)) incubated with $VGLUT_1$ ((a), (b)) or $VGLUT_2$ ((c), (d)) antibodies. ((a), (b)) $VGLUT_1$-IR nerve fibers are discretely observed in the glabrous (double arrowheads in (a), showing nerve fibers in close proximity to the epidermis) and the hairy skin (black double arrowheads in (b), showing the follicular neural network; white double arrowheads showing fibers lying in the basal membrane of the epidermis). ((c), (d)) Abundant $VGLUT_2$-IR nerve fibers are detected in the glabrous (arrowheads in (c), showing nerve endings penetrating the epidermis) and the hairy skin (black double arrowheads in (d), showing the follicular neural network; white double arrowheads, showing nerve endings in the dermis and epidermis). Scale bars: 50 μm ((c) = (a); (b); (d)). *Figures (b) and (d) are reproduced in part, and with permission, from [54].*

or $VGLUT_2$, mainly due to lacking of reliable antibodies. However, free nerve endings in the mouse palatine mucosa expressing $VGLUT_3$ (as well as $VGLUT_1$ and $VGLUT_2$) in addition to their presence in corpuscular nerve endings and Merkel cells, have been reported [171]. On the contrary, the limited number of $VGLUT_3$-expressing colorectal [61] and urinary bladder [62] DRG neurons (identified in $VGLUT_3$-EGFP mice [66]) suggests that only few if any nerve endings containing this transporter reach those organs. In skin, peripheral nerve endings produced by $VGLUT_3$-expressing DRG neurons have been recently exposed by the use of transgenic mice where the reporter gene Tomato is expressed under the control of the $VGLUT_3$ promoter [65]. In this study, $VGLUT_3$-expressing DRG neurons were shown to terminate in the skin in two different fashions: (1) as C-low threshold mechanoreceptors forming longitudinal lanceolate endings around hairs, and (2) as epidermal free nerve endings [65]. The neuroanatomy of Tomato-positive fibers innervating visceral organs remains to be explored.

In accordance with the peptidergic nature of their parent DRG neurons, the great majority of nerve fibers innervating the colorectal mucosa in the mouse exhibit a high degree of coexpression of CGRP and $VGLUT_2$ [61]. This is in contrast to nerve fibers located in the myenteric plexus, where most $VGLUT_2$ and CGRP-IR fibers remained as different populations [61]. In support, nonpeptidergic $VGLUT_2$-containing varicosities have also been reported in the esophageal myenteric plexus of rat [161]. Since a small subpopulation of $VGLUT_2$-IR mouse colorectal DRG neurons is nonpeptidergic (~18%), it could be postulated that they selectively innervated the myenteric plexus. Alternatively, these nonpeptidergic $VGLUT_2$-IR nerve fibers in the myenteric plexus could derive from neurons in the LSC or the MPG, two major contributors of nerve fibers in the colorectum [79] and the urinary bladder [172]. However, only rarely $VGLUT_2$-IR neurons are observed in normal conditions in these ganglia [61, 62].

Finally, coexpression of VGLUTs in peripheral nerve endings has been shown for $VGLUT_1$ and $VGLUT_2$ in rat [164] and mouse [165] (but not in guinea pig [163]) IGLEs [173]. Also, Merkel cells in the rat sinus hair follicle express $VGLUT_2$ and often show colocalization with $VGLUT_1$ [169].

4. Expression of VGLUTs in the Spinal Cord

Thinly myelinated or unmyelinated low threshold Aδ- and C-fibers transmitting nociceptive information and terminating predominantly in the superficial layers (laminae I and II) of the spinal dorsal horn release glutamate [174, 175]. Local spinal cord neurons are also capable of synthesizing and utilizing glutamate as their major excitatory neurotransmitter [3, 176, 177]. However, dissecting the patterns of expression

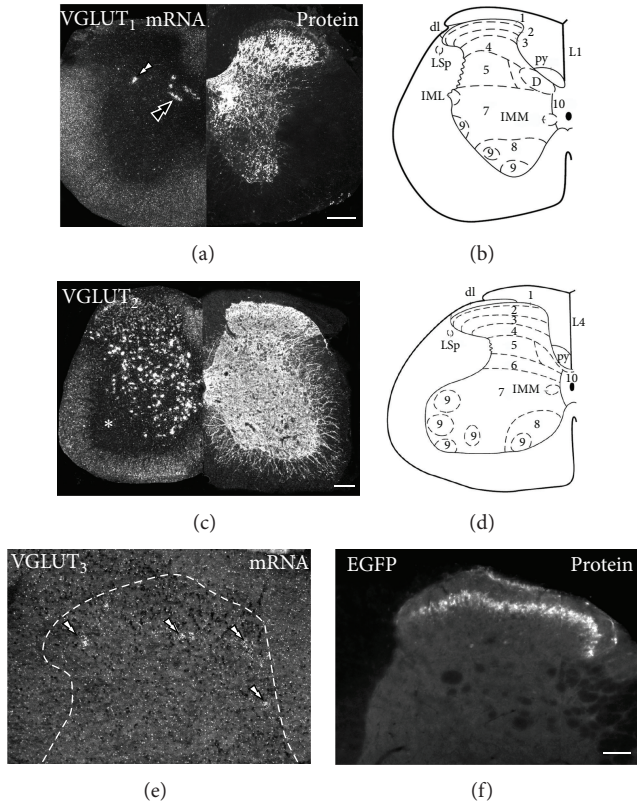


Figure 5: *VGLUTs protein and transcript expressions in the mouse spinal cord*. Dark-field ((a) and (c), left side; (e)) and immunofluorescence ((a) and (c), right side; (f)) photomicrographs of sections of the thoracolumbar and lumbar enlargement ((c), (e), (f)) of the spinal cord, incubated with $VGLUT_1$, $VGLUT_2$, or $VGLUT_3$ antisense riboprobes ((a), (c), (e), resp.) or $VGLUT_1$ (a), $VGLUT_2$ (c), or EGFP (f) antibodies. Schematic drawings of the thoracolumbar (b) and lumbar enlargement (d) of the spinal cord are provided as references for the laminae in the gray matter (taken from The Rat Brain in Stereotaxic Coordinates, Fourth Edition, George Paxinos and Charles Watson, 1998). (a) A discrete number of $VGLUT_1$ mRNA-positive NPs are detected in the dorsomedial aspect of the intermediate dorsal horn at thoracolumbar segments (black double arrowheads) and in more isolated fashion, in laminae IV-V of the dorsal horn (white double arrowhead). An abundant $VGLUT_1$-IR neuropil is also detected in the dorsal and ventral horns, being more intense in the deep dorsal horn and in area X and only weak in laminae I-II. (c) Abundant $VGLUT_2$ mRNA-positive NPs are detected in the lumbar enlargement of the spinal cord, encompassing both the dorsal and ventral horns. The $VGLUT_2$ mRNA signal in NPs in laminae II-III appears somewhat more diffuse than in deeper laminae. The areas occupied by laminae IX are, however, devoid of $VGLUT_2$-expressing NPs (asterisk). $VGLUT_2$-Li is abundant in the neuropil in the whole gray matter. ((e), (f)) Only few $VGLUT_3$ mRNA-positive NPs are detected in laminae III-IV of the dorsal horn (white double arrowheads in (e)). A modest EGFP-IR neuropil is also observed in laminae II (f). Scale bars: 200 μm ((a), (c)) 50 μm ((f) = (e)). *Figure (f) is reproduced in part, and with permission, from [66].*

and morphology of glutamatergic primary afferent terminals and spinal cord neurons from a "VGLUT perspective" has been challenging, mainly due to the failure of virtually all available VGLUT antibodies to produce immunohistochemical signal in the cell bodies of spinal cord neurons and the complex contribution of nerve terminals in the area (primary afferent versus local neurons, dendritic and axonal projections versus descending fibers). Nevertheless, the combined knowledge derived from immunohistochemical [30, 40, 46, 48–50, 54, 64, 66, 178–186] and *in situ* hybridization [43, 48, 51, 136, 187] studies allow today for a rather complete depiction of the VGLUT scenario in the spinal cord.

Thus, $VGLUT_1$- and $VGLUT_2$-Lis are easily detected in the neuropil of the gray matter in the spinal cord, although both transporters appear distributed differently between laminae (Figure 5). In rat [30, 40, 46, 48, 50] and mouse [54], $VGLUT_1$-Li is strong in laminae IIi–IV, medial laminae V/VI, dorsal lamina VII, and around lamina IX motoneurons. In contrast, $VGLUT_1$-Li is weak in laminae I, IIo, the lateral part of lamina V, the lateral spinal nucleus, and lamina VIII. Regarding $VGLUT_2$, studies in rat [30, 46, 48, 50] and mouse [54] exposed its abundant presence in laminae I and IIo, areas known to receive nociceptive fibers. Deeper in laminae IV-V, $VGLUT_2$-Li appears moderate but progressively increases towards the ventral horns. As for $VGLUT_3$, either through detection of weak $VGLUT_3$ and stronger EGFP immunohistochemical signals in transgenic mice, presence of the transporter has been shown in the neuropil of laminae I–IIi neuropil (Figure 5) [66]. The question is, how are these different immunohistochemical patterns generated?

DRG neurons are undoubted contributors of $VGLUT_1$-containing nerve fibers in the spinal cord. Varoqui et al. [30]

were the first in suggesting this and later confirmed by studies showing that transganglionically labelled primary afferent terminals in the dorsal horn of the spinal cord of rat expressed $VGLUT_1$- or $VGLUT_2$-Li [49]. Further support came from studies using dorsal rhizotomy, a procedure that completely blocks the central axonal transport of molecules produced by DRG neurons. Thus, dorsal rhizotomy dramatically, but not completely, reduces $VGLUT_1$-LI in the ventral horn (and to a lesser extent also in the dorsal horn), both in rat [46, 48, 50] and mouse [54].

However, the persistence of at least some $VGLUT_1$-Li after dorsal rhizotomy suggests additional sources, including: (1) primary afferents expressing this transporter and entering the spinal cord at levels higher and lower to the lesion and travelling certain distances before contacting their second order neurons; (2) local dorsal horn neurons; (3) brainstem- [48, 178] and cortical-derived [26, 178, 179] nerve terminals. Supporting the local origin, oligo- and riboprobe, radioactive or nonradioactive *in situ* hybridization studies by Kullander et al. [187] in neonatal mice, and Oliveira et al. [48] and Llewellyn-Smith et al. [40] in adult rats, revealed a few large $VGLUT_1$ mRNA-positive neurons in the dorsomedial part of the intermediate zone of the dorsal horn of the thoracic spinal cord, resembling dorsal nucleus of Clarke neurons. Studies in adult rats also suggested presence of $VGLUT_1$ mRNA-positive neurons in the lamina I of the dorsal horn [51], as well as in motoneurons (Figure 5) [43, 51]. More recently, in a study in adult mouse, we confirmed the expression of $VGLUT_1$ mRNA in a small group of neurons also resembling the dorsal nucleus of Clarke, and in occasional deep dorsal horn neurons at thoracolumbar levels [136]. Other spinal neurons, including motoneurons or superficial dorsal horn neurons, lacked $VGLUT_1$ [136]. The presence of $VGLUT_1$ in the dorsal nucleus of Clarke, known to be the origin of the spinocerebellar pathways [188], is supported by the detection of abundant $VGLUT_1$-Li in nerve fibers terminating in the anterior and posterior zones of the cerebellum [180], normally receiving spinocerebellar mossy fibers [181].

With one exception in the rat showing an ipsilateral decrease [46], dorsal rhizotomy appears unable to alter the immunoreactivity of $VGLUT_2$ in the dorsal horn of both rat [48, 50] and mouse [54]. This suggested that most if not all $VGLUT_2$-Li was dependent on local or supraspinal neurons. In fact, supraspinal sources of $VGLUT_2$ in the spinal cord have been recently demonstrated, as shown by their immunohistochemical presence in rubrospinal, vestibulospinal and reticulospinal tracts in rat [178]. However, a great proportion of $VGLUT_2$-Li in the spinal cord is likely dependent on numerous spinal cord neurons, as shown by their expression of $VGLUT_2$ transcript in the ventral and lateral aspects of the intermediate zone, in discrete parts of the ventral horns, and in the dorsal horn in rats [40, 48] and mice [136, 187] (Figure 5).

$VGLUT_2$ mRNA-positive neurons in the spinal cord likely belong to both the interneuron [48, 49] and projection neuron subpopulations [40, 48, 51, 136]. On one hand, interneurons in the rat spinal cord of rat expressing somatostatin, neurotensin, substance P, and/or enkephalin coexpress $VGLUT_2$ [49]. More recently, functionally identified excitatory interneurons in the rat have been shown to express $VGLUT_2$-Li [177, 182, 183]. On the other hand, the presence of $VGLUT_2$-Li in the large lemniscal and spinothalamic terminals to the ventral posterior thalamic nuclei in the rat [184] confirms that at least a number of neurons expressing $VGLUT_2$ mRNA in the rat and mouse dorsal horn are projection neurons. Moreover, and as pointed out above, coexpression of both $VGLUT_1$- and $VGLUT_2$-Lis in mossy fibers in the cerebellum [180] indicates that Clarke's nucleus projection neurons also express $VGLUT_2$. Finally, with the exception of one study in rat, suggesting that both $VGLUT_1$ and $VGLUT_2$ are expressed in motoneurons [43], other studies in rat [40, 48] and mouse [54, 136, 187] report that motoneurons, as well as neurons in area X, lack $VGLUT_2$, at least at the lumbar enlargement. However, motoneurons express other glutamatergic markers such as glutamate itself [185, 189] and/or EAAT-3 [185] and thus may utilize a yet undescribed VGLUT.

Is then the contribution of primary afferents to the $VGLUT_2$-Li in the dorsal horn of the spinal cord completely ruled out? The answer is no since $VGLUT_2$ has indeed been identified in transganglionically labelled primary afferents in the dorsal horn [49], and it modestly accumulates after dorsal root ligation (DRG side of the ligation) [54]. It is thus possible that low quantities of $VGLUT_2$ were transported by the central projections of DRG neurons and that the intense local- and supraspinal-dependent $VGLUT_2$-Li in the spinal cord neuropil acted as a masking factor, potentially explaining lack of changes after dorsal rhizotomy [46]. Interestingly, the neuropeptide tyrosine receptor type 1 (Y_1R), normally expressed by small primary afferent neurons, undergoes axonal transport and can be immunohistochemically detected in the dorsal horn, but its signal remains unaffected by dorsal rhizotomy [190]. In this case, and as discussed for $VGLUT_2$, the abundant expression of the Y_1R in local dorsal horn neurons appears to mask the expected decrease of the receptor after dorsal rhizotomy [190].

As for $VGLUT_3$, its modest immunohistochemical detection in the superficial dorsal horn has been shown to depend on DRG and to a lesser extent also supraspinal and spinal cord neurons. Thus, (1) dorsal rhizotomy in transgenic mice results in an almost complete disappearance of $VGLUT_3$-regulated EGFP-Li, normally observed in the superficial laminae of the dorsal horn, certifying its peripheral origin [66]; (2) Oliveira et al. [48], reported presence of a $VGLUT_3$-IR neuropil in the sympathetic intermediolateral column (often coexpressing serotonin), supporting their origin in the dorsal and median raphe nucleus, where such localization has already been demonstrated [32, 33]; and (3) $VGLUT_3$ protein [51, 136] and transcript [32, 51, 65, 136] have been demonstrated in the spinal cord of rat [32, 51] and mouse [65, 136], by means of RT-PCR [32] and western blot [51] in spinal cord extracts and by *in situ* hybridization in tissue sections [51, 65, 136]. More specifically, $VGLUT_3$-expressing neurons have been detected in neurons in the deep dorsal horn and some in the ventral horn of adult rats [51], in the superficial and deep dorsal horn of neonatal mice [65], and in the deep dorsal horn of adult mice [136] (Figure 5). Interestingly,

the $VGLUT_3$-expressing subpopulation of neurons in the superficial layers of the dorsal horn described by Lou et al. in the neonatal mouse [65] is not detected in the adult mouse [136], suggesting developmental regulation of the transporter.

The complex peptidergic versus nonpeptidergic representation of VGLUTs in DRG neurons is also observed in their spinal axonal terminations. The general consensus is that $VGLUT_2$ is often associated with peptidergic nerve terminals [46], whereas $VGLUT_1$ is hardly so [48]. Thus, Li et al. [46] reported that SP-Li is present in ~50% of the $VGLUT_2$-IR primary afferent boutons in laminae II [46]. In support, functional CGRP and AMPA receptors colocalize in single dorsal horn neurons, suggesting that these neurons may receive contacts from primary afferent terminals expressing peptides, glutamate, or both [191]. More importantly, AMPA receptor $GluR_2$-IR puncta can be seen in contact with over 90% of CGRP-IR primary afferent synaptic boutons [192]. However, Todd et al. [49] reported that peptidergic primary afferents in the rat, as well as nonpeptidergic C-fibers, exhibit low levels of $VGLUT_2$-Li or even lack either $VGLUT_1$ or $VGLUT_2$.

Finally, and as observed in mouse DRG neuronal bodies [54], $VGLUT_1$ and $VGLUT_2$ colocalization is also detected in a proportion of primary afferent varicosities in laminae III-IV and IX in the rat spinal cord [49, 50, 179], as well as in the nucleus of the solitary tract [186]. The appearance of these varicosities is described as being "…relatively large… and contained immunoreactivity that was intense for $VGLUT_1$ but weak for $VGLUT_2$" [50].

5. Expression of VGLUTs in Autonomic Ganglia

5.1. Sympathetic and Parasympathetic Ganglia. In normal conditions, LSC neurons do not express VGLUTs [60–62], whereas only occasional $VGLUT_2$-IR neurons are detected in naïve mouse MPG [61, 62]. However, $VGLUT_2$-IR fibers are found in the mouse LSC and the MPG, in the latter forming perineuronal baskets (Figure 3) [60–62], often but not exclusively, around TH-IR MPG neurons [60].

The $VGLUT_2$-IR baskets observed in MPGs appear greatly dependent on extrinsic sources, as demonstrated by their dramatic immunohistochemical disappearance after axotomy of the pelvic nerve [60]. A sympathetic or parasympathetic preganglionic origin [80] for these $VGLUT_2$-IR baskets has been ruled out due to their lack of coexpression with TH or VAChT, respectively. Alternatively, they could derive from primary afferent fibers in their way to pelvic organs and also running through the MPG [80, 193, 194]. In support, Aïoun and Rampin [195] have shown the ultrastructural coexistence of glutamate and large dense core vesicles; the latter typically loaded with peptides, in axons and terminals in the rat MPG. In the mouse, however, $VGLUT_2$-IR MPG baskets lack CGRP [60]. Nevertheless, as described above, many nonpeptidergic mouse visceral DRG neurons express $VGLUT_2$ [61, 62]. Whether $VGLUT_2$-IR nonpeptidergic DRG neurons are both the origin of the MPG baskets, as well as of the neuropil surrounding myenteric plexus neurons in the mouse colorectum (see Section 3.4), and participate in sensory-motor coupling remains to be demonstrated. Finally, one additional source could be viscerofugal neurons projecting towards the MPG, found in rat [196], guinea pig [197], and mouse [198]. Interestingly, we recently showed a small subpopulation of myenteric neurons expressing $VGLUT_2$ mRNA in the mouse colorectum [61] (see below).

5.2. Enteric Neurons. Most studies analyzing the expression of glutamate and glutamatergic markers in enteric neurons have focused on proximal rather than distal parts of the gut. Thus, enteric neurons containing immunohistochemically detectable glutamate have been described in the myenteric and submucosal plexuses of rat [168] and guinea pig ileum [168, 199], as well as in myenteric ganglia of the rat stomach [200]. More recently, a study in humans suggested that glutamate was present in large intestine submucosal and myenteric plexuses as well as in nerve fibers innervating the circular muscle layer [201], supporting earlier studies showing basal as well as stimulated (electrical and chemical) release of glutamate presumably from longitudinal muscle myenteric plexus neurons in the guinea pig ileum [202, 203].

As expected, VGLUTs are detected in enteric neurons in the gut. Thus, $VGLUT_1$ was found in cholinergic and nitrergic neurons in rat [161, 164] and mouse [165] esophageal myenteric plexus. $VGLUT_2$ was reported in neurons in the guinea pig, rat, mouse ileum [44, 204] and in rat [164] and mouse [162, 164] esophagus. Even in humans, all three VGLUTs appear to be present in the small intestine myenteric plexus neurons, interganglionic varicose fibers, and perisomatic puncta [205]. In the distal gut, lack of signal of $VGLUT_1$ and $VGLUT_2$ in enteric neurons of guinea pig rectum first suggested absence of glutamatergic enteric neurons, at least in this species [166]. In mouse, however, both protein and transcript of $VGLUT_2$ are found in a small number of colorectal myenteric plexus neurons, scattered throughout the plexus in contrast to $VGLUT_1$ [61] or $VGLUT_3$ (Figure 3), which appear to be absent. What the phenotype of $VGLUT_2$-expressing mouse myenteric plexus neurons is, remains to be established. However, glutamate and substance P or choline acetyltransferase colocalization was reported in small intestine enteric neurons of the guinea pig and rat [168].

6. Effects of Peripheral Nerve Injury or Tissue Inflammation on the Expression of VGLUTs

6.1. Sensory Ganglia and the Spinal Cord. Peripheral nerve injury [84, 207–209] as well as peripheral tissue inflammation [210–212] induces downregulation and upregulation of numerous molecules involved in a variety of functions that include survival, regeneration, and pain transmission in DRG and sympathetic ganglia neurons, as well as motoneurons in the spinal cord [84]. In line with such changes, Al-Ghoul et al. [213] reported an increase in the immunohistochemical levels of glutamate in the superficial layers of the dorsal horn after chronic loose ligation of the sciatic nerve, in parallel with the expected decrease of substance P and CGRP. Such

Table 5: Changes in the expression of VGLUTs in DRGs, spinal cord, and/or LSCs, upon peripheral nerve injury (axotomy of the sciatic nerve) or hindpaw inflammation.

Tissue	Species	Lesion type	Protein			mRNA		
			$VGLUT_1$	$VGLUT_2$	$VGLUT_3$	$VGLUT_1$	$VGLUT_2$	$VGLUT_3$
DRG	*Mouse*	Axotomy	▼▼ [54]	▼ [54]$^{\alpha}$	NE	No change [136]	No change [136]	▼ [136]
		Hind. inflam.	NE	NE	NE	No change [136]	No change [136]	No change [136]
	Rat	Axotomy	NE	NE	NE	NE	NE	NE
		Hind. inflam.	NE	NE	NE	NE	NE	NE
Spinal cord	*Mouse*	Axotomy	▼▼▼ (LII-VIII, IX) [54]	No change [54]	NE	No change [136]	No change [136]	No change [136]
		Hind. inflam.	NE	NE	NE	No change [136]	No change [136]	No change [136]
	Rat	Axotomy	▼▼▼ (LII-VIII-IX) [214]	NE	NE	NE	NE	NE
		Hind. inflam.	NE	NE	NE	NE	NE	NE
LSC	*Mouse*	Axotomy	No change [60]	▲ [60]	NE	No change [60]	▲ [60]	No change*
		Hind. inflam.	NE	NE	NE	NE	NE	NE
	Rat	Axotomy	NE	NE	NE	NE	NE	NE
		Hind. inflam.	NE	NE	NE	NE	NE	NE

Arrowhead up: upregulation; arrowhead down: downregulation; NE: not evaluated; α: plus an increase in $VGLUT_2$-LI in small neuron profiles; *unpublished data; tissue has been processed and NPs quantified according to references [54, 60–62].

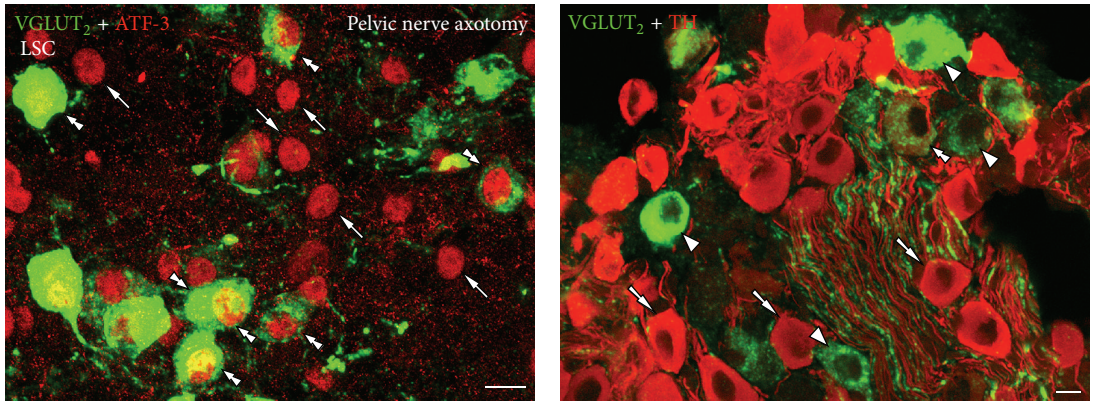
increase could be related to alterations in VGLUTs synthesis and axonal transport.

In fact, peripheral nerve lesions alter the expression of VGLUTs in primary afferent neurons (Table 5). Thus, Hughes et al. [214] were the first in demonstrating that axotomy of the sciatic nerve in rats induces depletion of $VGLUT_1$ protein in myelinated low threshold cutaneous and muscle mechanoreceptors terminating in the dorsal and ventral horns. It is now known that the depletion of $VGLUT_1$ in the spinal cord is mainly due to its reduced expression in DRG neurons, as shown in mouse [54]. Axotomy of the sciatic nerve in the mouse also reduces the numbers of $VGLUT_2$-IR DRG NPs, although a concomitant increase of $VGLUT_2$-Li was detected in a subpopulation of small DRG NPs [54]. However, and in contrast to $VGLUT_1$, changes in $VGLUT_2$ expression in DRGs do not translate into expected decreases/increases in $VGLUT_2$-Li at the lumbar levels of the spinal cord [54]. As explained for the lack of effect of dorsal rhizotomy on $VGLUT_2$-Li in Section 4, such a "failure" could relate to what appears to be a modest transport of $VGLUT_2$ from DRG neurons to the spinal cord [54], the abundant $VGLUT_2$-expression by local dorsal horn neurons [48–50, 53, 54, 136] and the presence of the transporter in descending pathways [178]. A similar "failure" to detect changes after axotomy of the sciatic nerve was previously reported in rat for the Y_1R in the superficial layers of the dorsal horn [215].

Somewhat surprisingly, the changes in immunohistochemical expression of $VGLUT_1$ and $VGLUT_2$ in DRG neurons appear to find no correlation in the expression of the corresponding transcripts. Thus, the number of $VGLUT_1$- or $VGLUT_2$ mRNA-positive DRG NPs remained unaltered in mice after a 7-day axotomy [136]. The only observed change was a modest downregulation in the number of $VGLUT_3$ mRNA-positive DRG NPs [136], although it is not known if axotomy also alters its protein expression. It is possible that differences in the techniques (*in situ* hybridization versus immunohistochemistry) and mouse strains (BalbC versus NMRI mice) between studies explained the discrepancy. However, it is also possible that differences between transcript and protein regulations in DRG neurons after peripheral nerve injury had biological meaning (see Section 7).

Finally, in the only published account so far, inflammation of the hindpaw (using a unilateral intradermal injection of Complete Freund's Adjuvant) failed to induce changes in the expression of $VGLUT_1$, $VGLUT_2$, or $VGLUT_3$ transcripts, both in DRG or spinal cord neurons [136]. Perhaps in these conditions, changes in expression and physiology of VGLUTs are more relevant in the axon and synaptic zones, where glutamate concentration and production is 2 to 3 times higher than in the cell body [19, 216, 217]. However, whether the inflammation of the hindpaw (or visceral organs) results in changes in VGLUT proteins in DRG neurons and their projections remains to be established.

6.2. Sympathetic Ganglia. Sympathetic neurons are profoundly affected by peripheral nerve injury [84, 101, 102, 209]. Thus, postganglionic axotomy of sympathetic nerves in cat LSC [218] or rodent superior cervical ganglion (SCG) [101, 102, 206] induces downregulation of neuropeptides such as CGRP [218] and NPY, as well as the noradrenergic marker TH [101, 102, 219, 220], and upregulation of galanin [101, 102, 218], VIP, SP [101, 102, 221], and the NPY Y_2-receptor [101, 102,



(a) (b)

Figure 6: *VGLUT$_2$ is upregulated in LSC neurons and occasionally coexpresses with TH*. Immunofluorescence photomicrographs of sections of the LSC of mouse after pelvic nerve axotomy, incubated with VGLUT$_2$ ((a), (b)) and ATF-3 (a) or TH (b) antibodies. ATF-3 is used as a marker of injured neurons. (a) Pelvic nerve axotomy results in *de novo* expression of ATF-3 in an abundant number of LSC NPs (arrows). Pelvic nerve axotomy also results in *de novo* upregulation of VGLUT$_2$, always coincidental with the upregulation of ATF-3 (double arrowheads). (b) Most VGLUT$_2$-IR LSC NPs observed after lesion lack TH (arrowheads), although occasional VGLUT$_2$/TH-IR NPs are detected (double arrowhead). Abundant TH-IR NPs are present in the LSC (arrows). Scale bars: 10 μm.

206]. Also in pigs, the axotomy of colonic nerves containing sympathetic fibers projecting from the LSC, results in the upregulation of galanin and somatostatin, paralleled by the downregulation of TH [222].

Only until recently, glutamate was not thought to be present in autonomic neurons [81]. In fact, apart from a few VGLUT$_2$-IR nerve fibers present in LSCs (more abundant in the stellate ganglion; unpublished results), VGLUTs are not normally expressed by neurons in these ganglia [60–62]. However, axotomy of either the pelvic (visceral) or the sciatic (nonvisceral) nerves results in the upregulation of VGLUT$_2$ in a number of mouse LSC neurons (Table 5, Figure 6) [60]. The lesion-induced VGLUT$_2$ plasticity appears to be selective in that parallel VGLUT$_1$ protein and transcript or VGLUT$_3$ mRNA upregulations are not observed [136]. The majority of VGLUT$_2$-IR LSC neurons detected after injury lack TH, suggesting a parallel downregulation of the noradrenergic marker (see above). However, some LSC neurons upregulating VGLUT$_2$ were shown to coexpress with TH, suggesting the possibility of glutamate and noradrenaline corelease [60] (Figure 6). Coexpression of VGLUT$_2$ [223–228] or glutamate [223] with TH (used as a marker for dopaminergic neurons) has been previously shown in rat [223, 225–228] brainstem and hypothalamus [228]. Alternatively, VGLUT$_2$ could be upregulated in LSC cholinergic (nonnoradrenergic) neurons [229], although this remains to be demonstrated.

The upregulation of VGLUT$_2$ in LSC neurons could represent *de novo* synthesis of transporter protein and transcript, or an increase from low, undetectable levels. The absence of VGLUT$_2$ (or other VGLUTs) transcript, using the sensitive riboprobe *in situ* hybridization techniques suggests the former hypothesis [60]. Considering the importance of VGLUTs for the uploading of glutamate into synaptic vesicles, it could be speculated that some LSC neurons, upon injury, acquire a glutamatergic phenotype, may release glutamate, and could potentially contribute to increased fast synaptic transmission and nociceptive mechanisms [60].

7. VGLUTs Modulation after Peripheral Nerve Injury—Implications to Glutamatergic Neurotransmission

While it is still not known exactly how many VGLUT copies are in each synaptic vesicle [35], it seems intuitive that changes in their expression after tissue insult should have profound consequences in glutamate loading. In fact, several studies where the expression of VGLUTs in synaptic vesicles is genetically manipulated suggest that the type and/or number of VGLUTs matter [19, 35]. For instance, the number of VGLUT copies in a given synaptic vesicle/neuron influences the amount and rate of vesicle loading, the size of glutamatergic quanta, and even the reserve pool of synaptic vesicles [158, 230–232].

In line with the above, the downregulation of VGLUT$_1$ [214] and VGLUT$_2$ proteins [54] observed in DRG neurons after sciatic nerve axotomy, while maintaining unaltered mRNA levels [136], may imply that these neurons sustain transcriptional levels of VGLUT$_1$ and VGLUT$_2$, in order to counteract the downregulation at the cell body level the latter resulting from increased axonal transport, protein use, and depletion at peripheral and central terminals [54]. Interestingly, axotomy of the sciatic nerve in rats results in a reduction in the number of synaptic vesicles in the central terminals of axotomized primary afferents [233], including peptidergic ones [234], suggesting increased fusion of clear synaptic vesicles (likely expressing VGLUTs) to the plasma membrane and glutamatergic release.

However, it should be noted that glutamatergic neurotransmission, from a synaptic vesicle point of view, is influenced by a variety of additional factors, comprising: (1) several steps to produce mature synaptic vesicles, mostly at the axons, although some may already happen in the soma [235]; (2) an active and tight regulation of presynaptic vesicle and transmitter recycling at the level of the synaptic cleft, to counteract depletion in situations of high activity [19, 216, 236]; (3) the extravesicular/cytoplasmic glutamate concentration (regulated by the enzyme glutaminase) 2 to 3 times higher in the terminals than in the cell body [217] and crucial in defining intravesicular glutamate content [19, 216]; (4) chloride conductance, along with the synaptic membrane potential, as also determining the glutamatergic content of synaptic vesicles and involving the participation of VGLUTs [237]; and (5) vesicular size, as it has been shown that there are different naturally occurring sizes that influence the quanta for different neurotransmitters, including glutamate [238]. How all these factors are challenged by peripheral nerve or tissue injury is not yet known. However, it has been shown that glutaminase is upregulated in rat DRG neurons during hindpaw inflammation and that its blockade results in reduced pain-like behaviour [239].

Altogether, nerve injury and/or inflammation and the accompanying pain, may result from changes in the expression of synaptic vesicles, associated proteins (including VGLUTs) and neuronal glutamatergic machinery in general, contributing to a fine "tuning" of pain mechanisms both at the synaptic as well as the cell body levels (see below).

8. VGLUTs, Proprioception, Pain, Survival, and Neuroprotection

8.1. Proprioception. Different studies in the peripheral and nervous systems suggest that proprioception is served by glutamatergic neurons expressing VGLUTs. This hypothesis is based on many of the observations in rodents presented above: (1) $VGLUT_1$ is expressed by a number of large and medium-sized DRG neurons [48, 51, 54] likely producing myelinated peripheral projections terminating in muscle and joint proprioceptors; (2) $VGLUT_1$-IR DRG neurons project fibers that have been morphologically characterized as proprioceptive [50, 214] towards the ventral horn and establish primary afferent-motoneuron contacts [40, 48–51, 54, 187, 214]; (3) many $VGLUT_1$-IR (likely proprioceptive) fibers also terminate in the deep dorsal horn, in the area occupied by Clarke's nucleus [48, 50, 54, 136, 187]; (4) Clarke's nucleus, composed by the second-order neurons giving rise to the spinocerebellar proprioceptive pathway [188] expresses $VGLUT_1$ [40, 48, 136, 187], and likely also $VGLUT_2$ [136, 180]; (5) $VGLUT_1$-Li (and also $VGLUT_2$) is detected in mossy fibers terminating in the anterior and posterior zones of the cerebellum [180], known to receive input from the spinocerebellar pathway described above [181]. In addition, and suggesting a role in the cortical control of motoneurons, pyramidal cells in the neocortex express $VGLUT_1$ mRNA [26], and numerous rat corticospinal tract nerve fibers terminating in the ventral horns exhibit $VGLUT_1$-Li [178, 179].

8.2. Nonvisceral Pain. The use of transgenic mice has exposed a central role for $VGLUT_2$ in the glutamatergic mechanisms associated to nonvisceral pain. Thus, heterozygote $VGLUT_2$ knock-out (KO) mice (homozygote mice experience perinatal death) exhibit impaired mechanical and cold allodynia after spared sciatic nerve injury, despite maintaining normal acute pain responses and increases in pain-like behaviour after inflammation of the hindpaw [240, 241]. Such behavioural patterns after partial $VGLUT_2$-KO originally suggested the involvement of the thalamus, whose neurons are richly provided with $VGLUT_2$ and presented with altered electrophysiological function in the transgenic mice [241]. However, mechanisms involving $VGLUT_2$ and pain appear to be relevant also at the DRG level. Thus, the selective deletion of $VGLUT_2$ in a subpopulation of TH- and $TRPV_1$-expressing neurons in mouse DRGs resulted in increased itch and decreased thermal pain sensitivity [155]. Interestingly, peripheral nerve injury results in $VGLUT_2$-Li increases in small mouse DRG neurons [54] (see Section 6.1), and it is possible that these were TRPV1-expressing (see Section 3.2). Thus, a hypothesis could be that activation of $TRPV_1$ in neurons upregulating $VGLUT_2$ during peripheral neuropathy may contribute to heightened peripheral and/or central release of glutamate, in the latter, resulting in the activation of nociceptive second-order projection neurons present in laminae I-II [242].

Selective deletion of $VGLUT_2$ in another subpopulation of DRG neurons expressing the sodium channel subtype Nav1.8 resulted in altered pain responses, including attenuated responses to intense mechanical stimuli [243]. Interestingly, most of these effects seem to be associated with reductions in glutamate release in the superficial layers of the dorsal horn, as shown by the reduced c-fos activation of local dorsal horn neurons upon noxious heat stimulation [155] or intraplantar injection of capsaicin [244]. Moreover, a reduction in spontaneous excitatory postsynaptic currents was observed in lamina II dorsal horn neurons in the $VGLUT_2$-KO-Nav1.8 mice [244].

Of the other known VGLUTs, $VGLUT_1$ appears mostly associated with proprioception (see above) and the transmission of tactile stimuli, as it has been proposed that many $VGLUT_1$-expressing primary afferent terminals in the superficial and deep dorsal horn correspond to low-threshold cutaneous mechanoreceptors, including those associated with the piloneural complex, in particular for nerve fibers terminating in lamina IIi of the superficial dorsal horn [49, 50, 214]. Deletion of $VGLUT_1$ in mice has no effect on nonvisceral pain behaviour [240].

On the contrary, $VGLUT_3$ is currently the center of debate. On one hand, deletion of $VGLUT_3$ in mice results in an increased threshold to intense noxious mechanical stimuli and reduced mechanical hypersensitivity to normally innocuous stimuli after tissue inflammation or nerve injury [66]. On the other hand, mice with deleted $VGLUT_3$ through knockout of the *runt* domain transcription factor Runx1, essential for the developmental control of unmyelinated sensory neurons (nociceptors, pruriceptors, and thermoceptors) [245] and also $VGLUT_3$ in sensory neurons [65], did not show

major changes in acute and chronic mechanical pain, with the exception of a modest increase in mechanical threshold after hindpaw carrageenan injection [65]. However, Seal et al. [66] utilized mice with global $VGLUT_3$ knockout (these mice also having deafness and rare nonconvulsive seizures), and thus, the behavioural effects observed in their study could also be influenced by deletion of the transporter in the spinal cord or other parts of the brain. With some limitations as well, the study by Lou et al. [65] analyzed neurons lacking $VGLUT_3$ but also Runx1, the latter influencing the expression of additional molecules in sensory neurons. Therefore, more research is needed to better understand the role of $VGLUT_3$ in acute and chronic nonvisceral pain.

Finally, the role of VGLUTs in nonvisceral nociception may also extend into the peripheral projections of DRG neurons. This is suggested by the increased glutamate (but not aspartate) levels in the hindpaw extracellular space, upon A- and/or C-fiber stimulation of the sciatic nerve, as well as the local injection of kainate or capsaicin [246]. Such a release of glutamate results in depolarizing effects on primary afferent C-fibers and the induction of pain-related behaviour of exogenously applied glutamate [246–249], likely acting on presynaptic glutamate (auto) receptors of various types [250–254]. The type of VGLUT involved in these mechanisms has not been defined. However, it is possible that $VGLUT_2$ was a main player, as suggested by its abundance in peripheral nerve endings in the skin [54].

8.3. Visceral Pain. The involvement of VGLUTs in visceral pain remains to be elucidated. The only study on the role of $VGLUT_2$ in visceral pain published so far found no differences between $VGLUT_2$-KO and littermate mice [240]. This is curious, especially when considering that the abundant numbers of $VGLUT_2$-expressing colorectal [61] and urinary bladder [62] neurons imply the likely colocalization of the transporter with several molecules associated with nociception, such as $TRPV_1$ [89], P_2X_3 [90], or the sodium channel NaV1.8 [91], only to cite a few. Thus, $TRPV_1$ is abundantly expressed in rat and mouse colorectal DRG neurons [76, 255] and has been associated to mechanisms of chronic visceral pain [256], and coexpression of $VGLUT_2$ with $TRPV_1$ has been previously reported in nerve fibers terminating in the mouse rectum [167]. In addition, P_2X_3, implicated in nociception [257], particularly in visceral organs [258, 259], is also expressed by a proportion of colorectal DRG neurons [77], and a role for Na(v)1.8 in visceral pain and hyperalgesia has also been reported [260]. Therefore, it would be expected that deletion of $VGLUT_2$ in so many neurons clearly prepared for nociception resulted in altered pain mechanisms. More research will be necessary to establish the extent to which $VGLUT_2$ participates in the physiopathology of visceral disorders associated with discomfort and pain.

Finally, preliminary experiments suggest that $VGLUT_3$ may not be involved in visceral hypersensitivity since its deletion does not alter the response to noxious mechanical distension of the colorectum, as compared to control mice (unpublished results). Accordingly, only a small percentage of $VGLUT_3$-expressing DRG neurons innervating the urinary bladder [62] or the colorectum (Figure 1) have been identified. However, an association between changes in the expression of $VGLUT_3$ in DRG neurons and visceral hyperalgesia in rats with *Trichinella Spiralis* infection has been proposed [64].

8.4. VGLUT-Expressing Sympathetic Neurons: Implications to Pain. The upregulation of $VGLUT_2$ in sympathetic neurons in the LSC (see Section 6.2) positions them as the new "kid on the block", acting as one additional contributor to peripherally released glutamate [60], along with primary afferents [48, 51, 53, 54, 54, 61, 62, 66, 154], and participating in processes of sympathetic-sensory neuron coupling [261, 262], also through glutamatergic neurotransmission.

In such scenario, glutamate released from primary afferent nerve terminals could act onto various types of glutamatergic receptors present in sympathetic postganglionic nerves [263–266], promoting an augmented release of noradrenaline and perhaps also glutamate. In turn, such "sympathetically" released glutamate could act on existing glutamatergic receptors in both visceral [267–270] and nonvisceral [175, 253, 271–275] primary afferent neurons, perpetuating a state of excitation in conditions such as inflammation [266] or nerve injury. More research will be necessary to: (1) assess the role of nerve injury-induced upregulation of $VGLUT_2$ in LSC neurons; (2) explore if its expression is also affected by other types of visceral organs pathological conditions (e.g., ulcerative colitis or interstitial cystitis); and (3) what is the consequence of deleting $VGLUT_2$ on autonomically driven nociceptive mechanisms.

8.5. VGLUTs, Survival, and Neuroprotection. The increased expression of $VGLUT_2$ in LSC neurons appears tightly related to the occurrence of axonal damage, as shown by the concomitant upregulation of the activating transcription factor 3 (ATF-3) [60] (Figure 6), a classical marker of damaged peripheral axons [276]. Axotomy of the rat or mouse superior cervical ganglion postganglionic axons also results in *de novo* ATF-3 expression [277], although it is not known if $VGLUT_2$ is upregulated in these neurons. Interestingly, ATF-3 [278], as well as the classical nerve growth factor (NGF), are central to mechanisms of nerve regeneration, and neuronal survival [80, 209]. Whether the upregulated $VGLUT_2$ (and potentially also glutamate) had a role in the survival and regeneration of LSC neurons remains to be established. However, several studies support such concept. Thus, $VGLUT_2$-Li is present in neurons migrating from the olfactory placode towards the forebrain in the developing rat brain, gradually decreasing toward adulthood [279]. Moreover, an association between the expression of $VGLUT_2$ protein in mesencephalic dopaminergic (DA) neurons and their formation of synaptic junctions in the nucleus accumbens was demonstrated in rat [280]. Furthermore, conditional knockout of $VGLUT_2$ results in reduced growth and survival of mesencephalic DA neurons, decrease in the density of DA innervation in the nucleus accumbens, reduced activity-dependent DA release, and impaired motor behaviour [281]. Thus, despite

the established concept that excessive activation of glutamatergic receptors results in neurotoxicity [282], in certain cases glutamate could have the opposite effect and contribute to survival and neuroprotection [283].

Interestingly, VGLUTs may also serve a role in development and neuroprotection in DRG neurons, as suggested in a recent study showing that glutamate release is essential to the development, maintenance, and sensory function of the piloneural mechanoreceptor, with $VGLUT_2$ being a key player [284].

9. Could VGLUTs Become Pharmacological Targets for the Control of Pain?

Throughout this review, we have highlighted the current knowledge on VGLUTs in peripheral neurons and the spinal cord, their regulation by tissue injury, and their involvement in sensation and pain. The abundance of $VGLUT_2$ in the periphery implies a fundamental role in glutamatergic physiology, even though the more discrete expression of $VGLUT_1$ and $VGLUT_3$ also suggests specific roles in select groups of DRG neurons. Data in transgenic mice, where a 50% reduction in $VGLUT_2$ [155, 240, 241, 243] or total ablation of $VGLUT_3$ [66] protein results in reduced/attenuated mechanical and cold hyperalgesia/allodynia after peripheral nerve injury or nonvisceral inflammation, while leaving unaffected other types of sensory processing, including acute nociception and inflammatory hyperalgesia, are compelling. Based on this knowledge, pharmacological blockade (total or partial) of VGLUT activity could efficiently reduce the amount of glutamate per vesicle, affect the size of glutamatergic quanta [241, 285, 286], and thus, attenuate glutamatergic neurotransmission, both at central and/or peripheral sites, resulting in the reduction of pain.

Exogenous VGLUT inhibitors, such as Chicago sky blue 6B (CSB6B), have been shown to inhibit the loading of glutamate into synaptic vesicles upon intracerebroventricular application [287], resulting in the inhibition of the methamphetamine induced hyperlocomotion and behavioural sensitization [288]. Interestingly, in an older study, Beirith et al. [289] evaluated the role of a systemically delivered CSB6B in animals receiving an intraplantar injection of glutamate and found that the use of the VGLUT inhibitor results in a considerable reduction of glutamate-induced nociception. However, the site of action of systemically applied CSB6B was not evaluated in that study, and thus, whether the inhibition of vesicular glutamate uptake occurs at peripheral nerve endings, spinal cord, supraspinal levels, or all of them remains to be established. Other exogenous compounds, including the dye Evans Blue or the Bengal Rose extract, have been described as VGLUT inhibitors and await further characterization [290, 291].

Endogenous VGLUT regulators also exist. Thus, fasting or diets with high lipidic and low glucose content (ketogenic diet), originally used to successfully reduce epileptic seizures [292], result in reduced pain and inflammation in juvenile and adult rats [292, 293]. Interestingly, the mechanisms of action proposed for ketogenic diets include decreases in the intracellular glutamatergic pool, as shown in cultured cerebellar granule neurons [294] and of glutamate uptake in synaptic vesicles by interference with the VGLUT chloride binding sites described in Section 7 [295]. However, the possible association of ketogenic diets, VGLUTs modulation, glutamate vesicular loading and pain mechanisms remain to be further established. Finally, different products of the kynurenine pathway in the metabolism of the amino acid tryptophan have been shown to exert antinociceptive roles after intraperitoneal administration in rats [296, 297], possibly through inhibition of VGLUTs activity [298].

Synthetic VGLUT inhibitors have been recently developed and their blocking action demonstrated *in vitro* [299, 300]. Unfortunately, these inhibitors do not discriminate between VGLUT types, and their role *in vivo* has yet to be determined. The development of selective VGLUT antagonists not only could help in dissecting the role of each VGLUT in *in vitro* and *in vivo* studies, but could also be therapeutically interesting, since regulation of the quantal size before fusion to the plasma membrane is emerging as an attractive approach to regulate the function of several neurotransmitters and as a tool to generate new pharmacological compounds [19, 301]. It is noteworthy that commercially available anticonvulsant agents such as gabapentin, lamotrigine, and riluzole limit glutamate release as part of their mechanisms of action and have been shown to be effective in reducing hyperalgesia in rats with neuropathy [302]. However, whether these agents act on VGLUTs to affect glutamate release is currently unknown.

The reduction of pain behaviour by topical targeting of peripheral glutamatergic mechanisms should also be considered. Thus, blockade of peripheral glutamatergic receptors emerge as an interesting therapeutic option [253, 270, 274, 303, 304], especially in view of the complex and serious CNS-driven side effects of systemically delivered glutamatergic receptor antagonists [303, 305, 306]. Likewise, inhibiting the peripheral synthesis of glutamate by targeting glutaminase bears promise [16]. In fact, glutaminase is upregulated in rat DRG neurons during inflammatory processes of the hindpaw [239], and its peripheral inhibition results in a reduction of the inflammation-induced hindpaw edema and of c-fos expression in laminae I-II of the dorsal horn of rats, as well as long-lasting analgesia [307]. Finally, and based on their abundant peripheral representation (especially for $VGLUT_2$), it is likely that challenging the activity of peripheral VGLUTs should also result in efficient modulation of glutamatergic neurotransmission.

10. Summary

In conclusion, this review has addressed various aspects relating to VGLUTs in visceral and nonvisceral DRGs, sympathetic neurons, and the spinal cord. When focusing on some of the functions of VGLUTs, the expression of $VGLUT_1$ in primary afferent nerves terminating in spinal areas such as those occupied by the dorsal nucleus of Clarke (also $VGLUT_1$-expressing) and motoneurons suggests a role in proprioception, whereas $VGLUT_2$, and to some extent

$VGLUT_3$, exhibits a robust association to nociception and pain. Moreover, the frequent coexpression of $VGLUT_2$ and CGRP supports the idea of corelease, and this could be relevant in processes of neurogenic inflammation. The *de novo* expression of $VGLUT_2$ in the LSC supports previously unexpected roles, such as sympathetic glutamatergic neurotransmission and/or survival and neuroprotection. Finally, the efficacy of the genetic deletion of $VGLUT_2$ (even if only half of what a neuron normally produces) and $VGLUT_3$ for the control of pain in rodents highlights the potential of these VGLUTs as potentially interesting targets for the development of new analgesic compounds. In such line of thought, it would be important to analyze the presence and distribution of VGLUTs in human peripheral nervous tissue and how do they react to tissue or nerve insult.

Abbreviations

ATF-3: Activating transcription factor, type 3
CGRP: Calcitonin gene related peptide
CNS: Central nervous system
DA: Dopamine
DRG: Dorsal root ganglion
EAAT: Excitatory amin oacid transporter
EGFP: Enhanced green fluorescent protein
GC: Granular cells
IB4: Isolectin B4
IGLEs: Intraganglionic laminar endings
IR: Immunoreactive
KO: Knock-out
L: Lumbar
Li: Like-immunoreactivity
LSC: Lumbar sympathetic chain
LSN: Lumbar splanchnic nerve
MPG: Major pelvic ganglion
NaV 1.8: Voltage dependent sodium channel, type 1.8
NGF: Nerve growth factor
P_2X_3: P2X purinoceptor 3
PN: Pelvic nerve
PNS: Peripheral nervous system
SCG: Superior cervical ganglion
TH: Tyrosine hydroxylase
TL: Thoracolumbar
$TRPA_1$: Transient receptor potential cation channel, subfamily A, member 1
$TRPV_1$: Transient receptor potential cation channel, subfamily V, member 1
VGLUT: Vesicular glutamate transporter
Y1R: Neuropeptide tyrosine receptor, type 1
Y2R: Neuropeptide tyrosine receptor, type 2.

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References

[1] P. Marmiroli and G. Cavaletti, "The glutamatergic neurotransmission in the central nervous system," *Current Medicinal Chemistry*, vol. 19, no. 9, pp. 1269–1276, 2012.

[2] A. H. Dickenson, V. Chapman, and G. M. Green, "The pharmacology of excitatory and inhibitory amino acid-mediated events in the transmission and modulation of pain in the spinal cord," *General Pharmacology*, vol. 28, no. 5, pp. 633–638, 1997.

[3] W. D. Willis Jr. and R. E. Coggeshall, *Primary Afferent Neurons and the Spinal Dorsal Horn*, vol. 1, Plenum Publishers, New York, NY, USA, 2004.

[4] J. Storm-Mathisen, A. K. Leknes, A. T. Bore et al., "First visualization of glutamate and GABA in neurones by immunocytochemistry," *Nature*, vol. 301, no. 5900, pp. 517–520, 1983.

[5] O. P. Ottersen and J. Storm-Mathisen, "Different neuronal localization of aspartate-like and glutamate-like immunoreactivities in the hippocampus of rat, guinea-pig and Senegalese baboon (Papio papio), with a note on the distribution of γ-aminobutyrate," *Neuroscience*, vol. 16, no. 3, pp. 589–606, 1985.

[6] J. Storm-Mathisen and O. P. Ottersen, "Immunocytochemistry of glutamate at the synaptic level," *Journal of Histochemistry and Cytochemistry*, vol. 38, no. 12, pp. 1733–1743, 1990.

[7] F. Fonnum, "Glutamate: a neurotransmitter in mammalian brain," *Journal of Neurochemistry*, vol. 42, no. 1, pp. 1–11, 1984.

[8] J. H. Laake, R. Torp, and O. P. Ottersen, "Ultrastructural immunocytochemical studies as a means of distinguishing between transmitter and non-transmitter glutamate," *Biochemical Society Transactions*, vol. 21, no. 1, pp. 45–49, 1993.

[9] L. H. Bergersen and V. Gundersen, "Morphological evidence for vesicular glutamate release from astrocytes," *Neuroscience*, vol. 158, no. 1, pp. 260–265, 2009.

[10] A. Wanaka, Y. Shiotani, H. Kiyama et al., "Glutamate-like immunoreactive structures in primary sensory neurons in the rat detected by a specific antiserum against glutamate," *Experimental Brain Research*, vol. 65, no. 3, pp. 691–694, 1987.

[11] G. Battaglia and A. Rustioni, "Coexistence of glutamate and substance P in dorsal root ganglion neurons of the rat and monkey," *Journal of Comparative Neurology*, vol. 277, no. 2, pp. 302–312, 1988.

[12] K. N. Westlund, D. L. McNeill, and R. E. Coggeshall, "Glutamate immunoreactivity in rat dorsal root axons," *Neuroscience Letters*, vol. 96, no. 1, pp. 13–17, 1989.

[13] M. A. Kai-Kai and R. Howe, "Glutamate-immunoreactivity in the trigeminal and dorsal root ganglia, and intraspinal neurons and fibres in the dorsal horn of the rat," *Histochemical Journal*, vol. 23, no. 4, pp. 171–179, 1991.

[14] J. R. Keast and T. M. Stephensen, "Glutamate and aspartate immunoreactivity in dorsal root ganglion cells supplying visceral and somatic targets and evidence for peripheral axonal

transport," *Journal of Comparative Neurology*, vol. 424, no. 4, pp. 577–587, 2000.

[15] T. Kaneko, H. Akiyama, I. Nagatsu, and N. Mizuno, "Immunohistochemical demonstration of glutaminase in catecholaminergic and serotoninergic neurons of rat brain," *Brain Research*, vol. 507, no. 1, pp. 151–154, 1990.

[16] K. E. Miller, E. M. Hoffman, M. Sutharshan, and R. Schechter, "Glutamate pharmacology and metabolism in peripheral primary afferents: physiological and pathophysiological mechanisms," *Pharmacology and Therapeutics*, vol. 130, no. 3, pp. 283–309, 2011.

[17] T. Hökfelt, O. Johansson, and M. Goldstein, "Chemical anatomy of the brain," *Science*, vol. 225, no. 4668, pp. 1326–1334, 1984.

[18] N. C. Danbolt, "Glutamate uptake," *Progress in Neurobiology*, vol. 65, no. 1, pp. 1–105, 2001.

[19] R. H. Edwards, "The neurotransmitter cycle and quantal size," *Neuron*, vol. 55, no. 6, pp. 835–858, 2007.

[20] J. Broman, B. Hassel, E. Rinvik, and O. P. Ottersen, "Biochemistry and anatomy of transmitter glutamate," in *Handbook of Chemical Neuroanatomy*, O. P. Ottersen and J. Storm-Mathisen, Eds., pp. 1–44, Elsevier, Amsterdam, The Netherlands, 2000.

[21] B. Ni, P. R. Rosteck Jr., N. S. Nadi, and S. M. Paul, "Cloning and expression of a cDNA encoding a brain-specific Na+-dependent inorganic phosphate cotransporter," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 12, pp. 5607–5611, 1994.

[22] E. E. Bellocchio, R. J. Reimer, R.T. Fremeau Jr., and R. H. Edwards, "Uptake of glutamate into synaptic vesicles by an inorganic phosphate transporter," *Science*, vol. 289, no. 5481, pp. 957–960, 2000.

[23] S. Takamori, J. S. Rhec, C. Rosenmund, and R. Jahn, "Identification of a vesicular glutamate transporter that defines a glutamatergic phenotype in neurons," *Nature*, vol. 407, no. 6801, pp. 189–194, 2000.

[24] Y. Aihara, H. Mashima, H. Onda et al., "Molecular cloning of a novel brain-type Na+-dependent inorganic phosphate cotransporter," *Journal of Neurochemistry*, vol. 74, no. 6, pp. 2622–2625, 2000.

[25] L. Bai, H. Xu, J. F. Collins, and F. K. Ghishan, "Molecular and functional analysis of a novel neuronal vesicular glutamate transporter," *The Journal of Biological Chemistry*, vol. 276, no. 39, pp. 36764–36769, 2001.

[26] R. T. Fremeau Jr., M. D. Troyer, I. Pahner et al., "The expression of vesicular glutamate transporters defines two classes of excitatory synapse," *Neuron*, vol. 31, no. 2, pp. 247–260, 2001.

[27] M. Hayashi, M. Otsuka, R. Morimoto et al., "Differentiation-associated Na+-dependent inorganic phosphate cotransporter (DNPI) is a vesicular glutamate transporter in endocrine glutamatergic systems," *The Journal of Biological Chemistry*, vol. 276, no. 46, pp. 43400–43406, 2001.

[28] E. Herzog, G. C. Bellenchi, C. Gras et al., "The existence of a second vesicular glutamate transporter specifies subpopulations of glutamatergic neurons," *The Journal of Neuroscience*, vol. 21, no. 22, p. RC181, 2001.

[29] S. Takamori, J. S. Rhee, C. Rosenmund, and R. Jahn, "Identification of differentiation-associated brain-specific phosphate transporter as a second vesicular glutamate transporter (VGLUT2)," *The Journal of Neuroscience*, vol. 21, no. 22, p. RC182, 2001.

[30] H. Varoqui, M. K.-H. Schäfer, H. Zhu, E. Weihe, and J. D. Erickson, "Identification of the differentiation-associated Na+/PI transporter as a novel vesicular glutamate transporter expressed in a distinct set of glutamatergic synapses," *The Journal of Neuroscience*, vol. 22, no. 1, pp. 142–155, 2002.

[31] R. T. Fremeau Jr., J. Burman, T. Qureshi et al., "The identification of vesicular glutamate transporter 3 suggests novel modes of signaling by glutamate," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 22, pp. 14488–14493, 2002.

[32] C. Gras, E. Herzog, G. C. Bellenchi et al., "A third vesicular glutamate transporter expressed by cholinergic and serotoninergic neurons," *The Journal of Neuroscience*, vol. 22, no. 13, pp. 5442–5451, 2002.

[33] M. K.-H. Schäfer, H. Varoqui, N. Defamie, E. Weihe, and J. D. Erickson, "Molecular cloning and functional identification of mouse vesicular glutamate transporter 3 and its expression in subsets of novel excitatory neurons," *The Journal of Biological Chemistry*, vol. 277, no. 52, pp. 50734–50748, 2002.

[34] Y. Moriyama and A. Yamamoto, "Glutamatergic chemical transmission: look! here, there, and anywhere," *The Journal of Biochemistry*, vol. 135, no. 2, pp. 155–163, 2004.

[35] S. Takamori, "VGLUTs: 'Exciting' times for glutamatergic research?" *Neuroscience Research*, vol. 55, no. 4, pp. 343–351, 2006.

[36] M. Liguz-Lecznar and J. Skangiel-Kramska, "Vesicular glutamate transporters (VGLUTs): the three musketeers of glutamatergic system," *Acta Neurobiologiae Experimentalis*, vol. 67, no. 3, pp. 207–218, 2007.

[37] Å. Wallen-Mackenzie, H. Wootz, and H. Englund, "Genetic inactivation of the vesicular glutamate transporter 2 (VGLUT2) in the mouse: what have we learnt about functional glutamatergic neurotransmission?" *Upsala Journal of Medical Sciences*, vol. 115, no. 1, pp. 11–20, 2010.

[38] P. Kitzman, "VGLUT1 and GLYT2 labeling of sacrocaudal motoneurons in the spinal cord injured spastic rat," *Experimental Neurology*, vol. 204, no. 1, pp. 195–204, 2007.

[39] R. N. Ranson, R. M. Santer, and A. H. D. Watson, "Ageing reduces the number of vesicular glutamate transporter 2 containing immunoreactive inputs to identified rat pelvic motoneurons," *Experimental Gerontology*, vol. 42, no. 6, pp. 506–516, 2007.

[40] I. J. Llewellyn-Smith, C. L. Martin, N. M. Fenwick, S. E. Dicarlo, H. L. Lujan, and A. M. Schreihofer, "VGLUT1 and VGLUT2 innervation in autonomic regions of intact and transected rat spinal cord," *Journal of Comparative Neurology*, vol. 503, no. 6, pp. 741–767, 2007.

[41] J. Zhou, N. Nannapaneni, and S. Shore, "Vessicular glutamate transporters 1 and 2 are differentially associated with auditory nerve and spinal trigeminal inputs to the cochlear nucleus," *Journal of Comparative Neurology*, vol. 500, no. 4, pp. 777–787, 2007.

[42] H.-Y. Zhou, S.-R. Chen, H. Chen, and H.-L. Pan, "The glutamatergic nature of TRPV1-expressing neurons in the spinal dorsal horn," *Journal of Neurochemistry*, vol. 108, no. 1, pp. 305–318, 2009.

[43] E. Herzog, M. Landry, E. Buhler et al., "Expression of vesicular glutamate transporters, VGLUT1 and VGLUT2, in cholinergic spinal motoneurons," *European Journal of Neuroscience*, vol. 20, no. 7, pp. 1752–1760, 2004.

[44] Q. Tong, J. Ma, and A. L. Kirchgessner, "Vesicular glutamate transporter 2 in the brain-gut axis," *NeuroReport*, vol. 12, no. 18, pp. 3929–3934, 2001.

[45] J.-L. Li, K.-H. Xiong, Y.-L. Dong, F. Fujiyama, T. Kaneko, and N. Mizuno, "Vesicular glutamate transporters, VGluT1 and VGluT2, in the trigeminal ganglion neurons of the rat, with special reference to coexpression," *Journal of Comparative Neurology*, vol. 463, no. 2, pp. 212–220, 2003.

[46] J.-L. Li, F. Fujiyama, T. Kaneko, and N. Mizuno, "Expression of vesicular glutamate transporters, VGluT1 and VGluT2, in axon terminals of nociceptive primary afferent fibers in the superficial layers of the medullary and spinal dorsal horns of the rat," *Journal of Comparative Neurology*, vol. 457, no. 3, pp. 236–249, 2003.

[47] M. J. Olave and D. J. Maxwell, "Axon terminals possessing the α2c-adrenergic receptor in the rat dorsal horn are predominantly excitatory," *Brain Research*, vol. 965, no. 1-2, pp. 269–273, 2003.

[48] A. L. R. Oliveira, F. Hydling, E. Olsson et al., "Cellular localization of three vesicular glutamate transporter mRNAs and proteins in rat spinal cord and dorsal root ganglia," *Synapse*, vol. 50, no. 2, pp. 117–129, 2003.

[49] A. J. Todd, D. I. Hughes, E. Polgár et al., "The expression of vesicular glutamate transporters VGLUT1 and VGLUT2 in neurochemically defined axonal populations in the rat spinal cord with emphasis on the dorsal horn," *European Journal of Neuroscience*, vol. 17, no. 1, pp. 13–27, 2003.

[50] F. J. Alvarez, R. M. Villalba, R. Zerda, and S. P. Schneider, "Vesicular glutamate transporters in the spinal cord, with special reference to sensory primary afferent synapses," *Journal of Comparative Neurology*, vol. 472, no. 3, pp. 257–280, 2004.

[51] M. Landry, R. Bouali-Benazzouz, S. El Mestikawy, P. Ravassard, and F. Nagy, "Expression of vesicular glutamate transporters in rat lumbar spinal cord, with a note on dorsal root ganglia," *Journal of Comparative Neurology*, vol. 468, no. 3, pp. 380–394, 2004.

[52] S.-X. Wu, Y. Koshimizu, Y.-P. Feng et al., "Vesicular glutamate transporter immunoreactivity in the central and peripheral endings of muscle-spindle afferents," *Brain Research*, vol. 1011, no. 2, pp. 247–251, 2004.

[53] J. L. Morris, P. König, T. Shimizu, P. Jobling, and I. L. Gibbins, "Most peptide-containing sensory neurons lack proteins for exocytotic release and vesicular transport of glutamate," *Journal of Comparative Neurology*, vol. 483, no. 1, pp. 1–16, 2005.

[54] P. Brumovsky, M. Watanabe, and T. Hökfelt, "Expression of the vesicular glutamate transporters-1 and -2 in adult mouse dorsal root ganglia and spinal cord and their regulation by nerve injury," *Neuroscience*, vol. 147, no. 2, pp. 469–490, 2007.

[55] L. Pawson, A. K. Pack, and S. J. Bolanowski, "Possible glutaminergic interaction between the capsule and neurite of Pacinian corpuscles," *Somatosensory and Motor Research*, vol. 24, no. 1-2, pp. 85–95, 2007.

[56] I. Pintelon, I. Brouns, I. de Proost, F. van Meir, J.-P. Timmermans, and D. Adriaensen, "Sensory receptors in the visceral pleura: neurochemical coding and live staining in whole mounts," *American Journal of Respiratory Cell and Molecular Biology*, vol. 36, no. 5, pp. 541–551, 2007.

[57] E. Polgár, K. M. Al-Khater, S. Shehab, M. Watanabe, and A. J. Todd, "Large projection neurons in lamina I of the rat spinal cord that lack the neurokinin 1 receptor are densely innervated by VGLUT2-containing axons and possess GluR4-containing AMPA receptors," *The Journal of Neuroscience*, vol. 28, no. 49, pp. 13150–13160, 2008.

[58] S. B. Mazzone and A. E. McGovern, "Immunohistochemical characterization of nodose cough receptor neurons projecting to the trachea of guinea pigs," *Cough*, vol. 4, no. 1, article 9, 2008.

[59] D. M. Hegarty, K. Tonsfeldt, S. M. Hermes, H. Helfand, and S. A. Aicher, "Differential localization of vesicular glutamate transporters and peptides in corneal afferents to trigeminal nucleus caudalis," *Journal of Comparative Neurology*, vol. 518, no. 17, pp. 3557–3569, 2010.

[60] P. R. Brumovsky, K. B. Seroogy, K. H. Lundgren, M. Watanabe, T. Hökfelt, and G. F. Gebhart, "Some lumbar sympathetic neurons develop a glutamatergic phenotype after peripheral axotomy with a note on VGLUT2-positive perineuronal baskets," *Experimental Neurology*, vol. 230, no. 2, pp. 258–272, 2011.

[61] P. R. Brumovsky, D. R. Robinson, J.-H. La et al., "Expression of vesicular glutamate transporters type 1 and 2 in sensory and autonomic neurons innervating the mouse colorectum," *Journal of Comparative Neurology*, vol. 519, no. 16, pp. 3346–3366, 2011.

[62] P. R. Brumovsky, R. P. Seal, K. H. Lundgren, K. B. Seroogy, M. Watanabe, and G. F. Gebhart, "Expression of vesicular glutamate transporters in sensory and autonomic neurons innervating the mouse bladder," *The Journal of Urology*, vol. 189, pp. 2342–2349, 2012.

[63] S. K. Paik, S. K. Kim, S. J. Choi, E. S. Yang, S. H. Ahn, and Y. C. Bae, "Vesicular glutamate transporters in axons that innervate the human dental Pulp," *Journal of Endodontics*, vol. 38, no. 4, pp. 470–474, 2012.

[64] C.-Q. Yang, Y.-Y. Wei, Y.-X. Leng et al., "Vesicular glutamate transporter-3 contributes to visceral hyperalgesia induced by trichinella spiralis infection in rats," *Digestive Diseases and Sciences*, vol. 57, pp. 865–872, 2011.

[65] S. Lou, B. Duan, L. Vong, B. B. Lowell, and Q. Ma, "Runx1 controls terminal morphology and mechanosensitivity of VGLUT3-expressing C-mechanoreceptors," *The Journal of Neuroscience*, vol. 33, pp. 870–882, 2013.

[66] R. P. Seal, X. Wang, Y. Guan et al., "Injury-induced mechanical hypersensitivity requires C-low threshold mechanoreceptors," *Nature*, vol. 462, no. 7273, pp. 651–655, 2009.

[67] V. Montana, Y. Ni, V. Sunjara, X. Hua, and V. Parpura, "Vesicular glutamate transporter-dependent glutamate release from astrocytes," *The Journal of Neuroscience*, vol. 24, no. 11, pp. 2633–2642, 2004.

[68] E. Anlauf and A. Derouiche, "Astrocytic exocytosis vesicles and glutamate: a high-resolution immunofluorescence study," *GLIA*, vol. 49, no. 1, pp. 96–106, 2005.

[69] Y. Ni, E. B. Malarkey, and V. Parpura, "Vesicular release of glutamate mediates bidirectional signaling between astrocytes and neurons," *Journal of Neurochemistry*, vol. 103, no. 4, pp. 1273–1284, 2007.

[70] D. Li, K. Herault, K. Silm et al., "Lack of evidence for vesicular glutamate transporter expression in mouse astrocytes," *The Journal of Neuroscience*, vol. 33, pp. 4434–4455, 2013.

[71] G. Gómez-Lira, M. Lamas, H. Romo-Parra, and R. Gutiérrez, "Programmed and induced phenotype of the hippocampal granule cells," *The Journal of Neuroscience*, vol. 25, no. 30, pp. 6939–6946, 2005.

[72] G. J. Bennett, "Animal models of pain," in *Methods in Pain Research*, L. Kruger, Ed., pp. 67–92, CRC Press, Boca Raton, Fla, USA, 2001.

[73] D. R. Robinson and G. F. Gebhart, "Inside information: the unique features of visceral sensation," *Molecular Interventions*, vol. 8, no. 5, pp. 242–253, 2008.

[74] S. J. Brookes, N. J. Spencer, M. Costa, and V. P. Zagorodnyuk, "Extrinsic primary afferent signalling in the gut," *Nature Reviews Gastroenterology & Hepatology*, vol. 10, pp. 286–296, 2013.

[75] H.-R. Berthoud and W. L. Neuhuber, "Functional and chemical anatomy of the afferent vagal system," *Autonomic Neuroscience*, vol. 85, no. 1–3, pp. 1–17, 2000.

[76] J. A. Christianson, R. J. Traub, and B. M. Davis, "Differences in spinal distribution and neurochemical phenotype of colonic afferents in mouse and rat," *Journal of Comparative Neurology*, vol. 494, no. 2, pp. 246–259, 2006.

[77] D. R. Robinson, P. A. Mcnaughton, M. L. Evans, and G. A. Hicks, "Characterization of the primary spinal afferent innervation of the mouse colon using retrograde labelling," *Neurogastroenterology and Motility*, vol. 16, no. 1, pp. 113–124, 2004.

[78] L. A. Blackshaw, S. J. H. Brookes, D. Grundy, and M. Schemann, "Sensory transmission in the gastrointestinal tract," *Neurogastroenterology and Motility*, vol. 19, no. 1, pp. 1–19, 2007.

[79] J. B. Furness, "The organisation of the autonomic nervous system: peripheral connections," *Autonomic Neuroscience*, vol. 130, no. 1-2, pp. 1–5, 2006.

[80] J. R. Keast, "Plasticity of pelvic autonomic ganglia and urogenital innervation," *International Review of Cytology*, vol. 248, pp. 141–208, 2006.

[81] G. Burnstock, "Autonomic neurotransmission: 60 years since Sir Henry Dale," *Annual Review of Pharmacology and Toxicology*, vol. 49, pp. 1–30, 2009.

[82] J. B. Furness, C. Jones, K. Nurgali, and N. Clerc, "Intrinsic primary afferent neurons and nerve circuits within the intestine," *Progress in Neurobiology*, vol. 72, no. 2, pp. 143–164, 2004.

[83] S. B. McMahon and J. V. Priestley, "Nociceptor plasticity," in *The Neurobiology of Pain*, S. P. Hunt and M. Koltzenburg, Eds., pp. 35–64, Oxford University Press, Oxford, UK, 2005.

[84] P. R. Brumovsky, M. J. Villar, and T. Hökfelt, "Retrograde cellular changes in primary afferent and sympathetic neurons after nerve injury," in *Encyclopedia of Pain*, R. Schmidt and G. F. Gebhart, Eds., Springer, Berlin, Germany, 2013.

[85] E. Bergman, K. Carlsson, A. Liljeborg, E. Manders, T. Hökfelt, and B. Ulfhake, "Neuropeptides, nitric oxide synthase and GAP-43 in B4-binding and RT97 immunoreactive primary sensory neurons: normal distribution pattern and changes after peripheral nerve transection and aging," *Brain Research*, vol. 832, no. 1-2, pp. 63–83, 1999.

[86] M. Zwick, D. C. Molliver, J. Lindsay et al., "Transgenic mice possessing increased numbers of nociceptors do not exhibit increased behavioral sensitivity in models of inflammatory and neuropathic pain," *Pain*, vol. 106, no. 3, pp. 491–500, 2003.

[87] P. Brumovsky, M. J. Villar, and T. Hökfelt, "Tyrosine hydroxylase is expressed in a subpopulation of small dorsal root ganglion neurons in the adult mouse," *Experimental Neurology*, vol. 200, no. 1, pp. 153–165, 2006.

[88] P. Brumovsky, D. Stanic, S. Shuster, H. Herzog, M. Villar, and T. Hökfelt, "Neuropeptide Y2 receptor protein is present in peptidergic and nonpeptidergic primary sensory neurons of the mouse," *Journal of Comparative Neurology*, vol. 489, no. 3, pp. 328–348, 2005.

[89] M. J. Caterina, "Transient receptor potential ion channels as participants in thermosensation and thermoregulation," *The American Journal of Physiology: Regulatory Integrative and Comparative Physiology*, vol. 292, no. 1, pp. R64–R76, 2007.

[90] M. F. Jarvis, "Contributions of P_2X_3 homomeric and heteromeric channels to acute and chronic pain," *Expert Opinion on Therapeutic Targets*, vol. 7, no. 4, pp. 513–522, 2003.

[91] S. D. Dib-Hajj, T. R. Cummins, J. A. Black, and S. G. Waxman, "Sodium channels in normal and pathological pain," *Annual Review of Neuroscience*, vol. 33, pp. 325–347, 2010.

[92] G. Burnstock and J. N. Wood, "Purinergic receptors: their role in nociception and primary afferent neurotransmission," *Current Opinion in Neurobiology*, vol. 6, no. 4, pp. 526–532, 1996.

[93] T. Hökfelt, X. Zhang, Z. Q. Xu et al., "Cellular and synaptic mechanisms in transition of pain from acuto to chronic," in *Proceedings of the 8th World Congress on Pain, Progress in Pain Research and Management*, T. S. Jensen, J. A. Turner, and Z. Wiesenfeld-Hallin, Eds., pp. 133–153, IASP Press, Seattle, Wash, USA, 1997.

[94] X. J. Xu and Z. Wiesenfeld-Hallin, "Novel modulators in pain," in *The Pharmacology of Pain, Handbook of Experimental Neurology*, A. H. Dickenson and J. M. Hesson, Eds., pp. 211–234, Springer, Berlin, Germany, 1997.

[95] W. C. de Groat, M. Kawatani, M. B. Houston, M. Rutigliano, and S. Erdman, "Identification of neuropeptides in afferent pathways to the pelvic viscera of the cat," in *Organization of the Autonomic Nervous System: Central and Peripheral Mechanisms. Neurology and Neurobiology*, J. Ciriello, F. Calaresu L Renaud, and C. Polosa, Eds., pp. 81–90, A.R. Liss, New York, NY, USA, 1987.

[96] J. R. Keast and W. C. de Groat, "Segmental distribution and peptide content of primary afferent neurons innervating the urogenital organs and colon of male rats," *Journal of Comparative Neurology*, vol. 319, no. 4, pp. 615–623, 1992.

[97] D. L. H. Bennett, N. Dmietrieva, J. V. Priestley, D. Clary, and S. B. McMahon, "trkA, CGRP and IB4 expression in retrogradely labelled cutaneous and visceral primary sensory neurones in the rat," *Neuroscience Letters*, vol. 206, no. 1, pp. 33–36, 1996.

[98] H. F. Wang, P. Shortland, M. J. Park, and G. Grant, "Retrograde and transganglionic transport of horseradish peroxidase-conjugated cholera toxin B subunit, wheatgerm agglutinin and isolectin B4 from Griffonia simplicifolia I in primary afferent neurons innervating the rat urinary bladder," *Neuroscience*, vol. 87, no. 1, pp. 275–288, 1998.

[99] P. R. Brumovsky, J. H. La, C. J. McCarthy, T. Hökfelt, and G. F. Gebhart, "Dorsal root ganglion neurons innervating pelvic organs in the mouse express tyrosine hydroxylase," *Neuroscience*, vol. 223, pp. 77–91, 2012.

[100] L.-G. Elfvin, B. Lindh, and T. Hökfelt, "The chemical neuroanatomy of sympathetic ganglia," *Annual Review of Neuroscience*, vol. 16, pp. 471–507, 1993.

[101] R. E. Zigmond and Y. Sun, "Regulation of neuropeptide expression in sympathetic neurons. Paracrine and retrograde influences," *Annals of the New York Academy of Sciences*, vol. 814, pp. 181–197, 1997.

[102] M. Landry, K. Holmberg, X. Zhang, and T. Hökfelt, "Effect of axotomy on expression of NPY, galanin, and NPY Y1 and Y2 receptors in dorsal root ganglia and the superior cervical ganglion studied with double-labeling in situ hybridization and immunohistochemistry," *Experimental Neurology*, vol. 162, no. 2, pp. 361–384, 2000.

[103] M. Costa and S. H. Brookes, "Architecture of enteric neural circuits involved in intestinal motility," *European Review for Medical and Pharmacological Sciences*, vol. 12, no. 1, pp. 3–19, 2008.

[104] B. Rexed, "The cytoarchitectonic organization of the spinal cord in the cat," *The Journal of Comparative Neurology*, vol. 96, no. 3, pp. 414–495, 1952.

[105] T. J. Grudt and E. R. Perl, "Correlations between neuronal morphology and electro-physiological features in the rodent superficial dorsal horn," *Journal of Physiology*, vol. 540, no. 1, pp. 189–207, 2002.

[106] S. A. Prescott and Y. De Koninck, "Four cell types with distinctive membrane properties and morphologies in lamina I of the spinal dorsal horn of the adult rat," *Journal of Physiology*, vol. 539, no. 3, pp. 817–836, 2002.

[107] R. Morris, O. Cheunsuang, A. Stewart, and D. Maxwell, "Spinal dorsal horn neurone targets for nociceptive primary afferents: do single neurone morphological characteristics suggest how nociceptive information is processed at the spinal level," *Brain Research Reviews*, vol. 46, no. 2, pp. 173–190, 2004.

[108] P. Burgess and E. R. Perl, "Cutaneous mechanoreceptors and nociceptors," in *Cutaneous Mechanoreceptors and Nociceptors*, A. Iggo, Ed., pp. 29–78, Springer, Berlin, Germany, 1973.

[109] M. Zimmermann, "Neurophysiology of nociception," in *Neurophysiology of Nociception*, R. Porter, Ed., pp. 179–221, University Park, Baltimore, Md, USA, 1976.

[110] A. R. Light and E. R. Perl, "Spinal termination of functionally identified primary afferent neurons with slowly conducting myelinated fibers," *Journal of Comparative Neurology*, vol. 186, no. 2, pp. 133–150, 1979.

[111] S. Gobel, W. M. Falls, and E. Humphrey, "Morphology and synaptic connections of ultrafine primary axons in lamina I of the spinal dorsal horn: candidates for the terminal axonal arbors of primary neurons with unmyelinated (C) axons," *The Journal of Neuroscience*, vol. 1, no. 10, pp. 1163–1179, 1981.

[112] A. D. Craig and S. Mense, "The distribution of afferent fibers from the gastrocnemius-soleus muscle in the dorsal horn of the cat, as revealed by the transport of horseradish peroxidase," *Neuroscience Letters*, vol. 41, no. 3, pp. 233–238, 1983.

[113] A. D. Craig, B. Heppelmann, and H.-G. Schaible, "The projection of the medial and posterior articular nerves of the cat's knee to the spinal cord," *Journal of Comparative Neurology*, vol. 276, no. 2, pp. 279–288, 1988.

[114] C. Morgan, I. Nadelhaft, and W. C. de Groat, "The distribution of visceral primary afferents from the pelvic nerve to Lissauer's tract and the spinal gray matter and its relationship to the sacral parasympathetic nucleus," *Journal of Comparative Neurology*, vol. 201, no. 3, pp. 415–440, 1981.

[115] J. Ciriello and F. R. Calaresu, "Central projections of afferent renal fibers in the rat: an anterograde transport study of horseradish peroxidase," *Journal of the Autonomic Nervous System*, vol. 8, no. 3, pp. 273–285, 1983.

[116] I. Nadelhaft, J. Roppolo, C. Morgan, and W. C. de Groat, "Parasympathetic preganglionic neurons and visceral primary afferents in monkey sacral spinal cord revealed following application of horseradish peroxidase to pelvic nerve," *Journal of Comparative Neurology*, vol. 216, no. 1, pp. 36–52, 1983.

[117] S. Gobel and W. M. Falls, "Anatomical observations of horseradish peroxidase-filled terminal primary axonal arborizations in layer II of the substantia gelatinosa of Rolando," *Brain Research*, vol. 175, no. 2, pp. 335–340, 1979.

[118] Y. Sugiura, N. Terui, and Y. Hosoya, "Difference in distribution of central terminals between visceral and somatic unmyelinated (C) primary afferent fibers," *Journal of Neurophysiology*, vol. 62, no. 4, pp. 834–840, 1989.

[119] Y. Sugiura, C. L. Lee, and E. R. Perl, "Central projections of identified, unmyelinated (C) afferent fibers innervating mammalian skin," *Science*, vol. 234, no. 4774, pp. 358–361, 1986.

[120] F. Cruz, D. Lima, W. Zieglgansberger, and A. Coimbra, "Fine structure and synaptic architecture of HRP-labelled primary afferent terminations in lamina IIi of the rat dorsal horn," *Journal of Comparative Neurology*, vol. 305, no. 1, pp. 3–16, 1991.

[121] J. I. Nagy and S. P. Hunt, "The termination of primary afferents within the rat dorsal horn: evidence for rearrangement following capsaicin treatment," *Journal of Comparative Neurology*, vol. 218, no. 2, pp. 145–158, 1983.

[122] F. Cruz, D. Lima, and A. Coimbra, "Several morphological types of terminal arborizations of primary afferents in laminae I-II of the rat spinal cord, as shown after HRP labeling and Golgi impregnation," *Journal of Comparative Neurology*, vol. 261, no. 2, pp. 221–236, 1987.

[123] C. J. Woolf, "Central terminations of cutaneous mechanoreceptive afferents in the rat lumbar spinal cord," *Journal of Comparative Neurology*, vol. 261, no. 1, pp. 105–119, 1987.

[124] P. Shortland, C. J. Woolf, and M. Fitzgerald, "Morphology and somatotopic organization of the central terminals of hindlimb hair follicle afferents in the rat lumbar spinal cord," *Journal of Comparative Neurology*, vol. 289, no. 3, pp. 416–433, 1989.

[125] P. Shortland and C. J. Woolf, "Morphology and somatotopy of the central arborizations of rapidly adapting glabrous skin afferents in the rat lumbar spinal cord," *Journal of Comparative Neurology*, vol. 329, no. 4, pp. 491–511, 1993.

[126] S. Ramón y Cajal, "General principles, spinal cord, spinal ganglia, medulla and pons," in *Histology of the Nervous System of Man and Vertebrates*, pp. 247–249, Oxford University Press, Oxford, UK, 1899.

[127] A. Brown, *Organization in the Spinal Cord: The Anatomy and Physiology of Identified Neurones*, Springer, Verlin, France, 1981.

[128] H. J. Ralston III and D. D. Ralston, "The distribution of dorsal root axons to laminae IV, V, and VI of the macaque spinal cord: a quantitative electron microscopic study," *Journal of Comparative Neurology*, vol. 212, no. 4, pp. 435–448, 1982.

[129] F. Cervero and L. A. Connell, "Distribution of somatic and visceral primary afferent fibres within the thoracic spinal cord of the cat," *Journal of Comparative Neurology*, vol. 230, no. 1, pp. 88–98, 1984.

[130] C. LaMotte, "Distribution of the tract of Lissauer and the dorsal root fibers in the primate spinal cord," *Journal of Comparative Neurology*, vol. 172, no. 3, pp. 529–561, 1977.

[131] C. Molander and G. Grant, "Laminar distribution and somatotopic organization of primary afferent fibers from hindlimb nerves in the dorsal horn. A study by transganglionic transport of horseradish peroxidase in the rat," *Neuroscience*, vol. 19, no. 1, pp. 297–312, 1986.

[132] H. F. Wang, B. Robertson, and G. Grant, "Anterograde transport of horseradish-peroxidase conjugated isolectin B4 from Griffonia simplicifolia I in spinal primary sensory neurons of the rat," *Brain Research*, vol. 811, no. 1-2, pp. 34–39, 1998.

[133] W. Neuhuber, "The central projections of visceral primary afferent neurons of the inferior mesenteric plexus and hypogastric nerve and the location of the related sensory and preganglionic sympathetic cell bodies in the rat," *Anatomy and Embryology*, vol. 164, no. 3, pp. 413–425, 1982.

[134] I. Nadelhaft and A. M. Booth, "The location and morphology of preganglionic neurons and the distribution of visceral afferents from the rat pelvic nerve: a horseradish peroxidase study,"

Journal of Comparative Neurology, vol. 226, no. 2, pp. 238–245, 1984.

[135] C. N. Honda, "Visceral and somatic afferent convergence onto neurons near the central canal in the sacral spinal cord of the cat," *Journal of Neurophysiology*, vol. 53, no. 4, pp. 1059–1078, 1985.

[136] M. Malet, C. A. Vieytes, K. H. Lundgren et al., "Transcript expression of vesicular glutamate transporters in lumbar dorsal root ganglia and teh spinal cord of mice—effects of peripheral axotomy or hindpaw inflammation," *Neuroscience*, vol. 248, pp. 95–111, 2013.

[137] G. Scherrer, S. A. Low, X. Wang et al., "VGLUT2 expression in primary afferent neurons is essential for normal acute pain and injury-induced heat hypersensitivity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 51, pp. 22296–22301, 2010.

[138] J. H. La, E. S. Schwartz, and G. F. Gebhart, "Differences in the expression of transient receptor potential channel V1, transient receptor potential channel A1 and mechanosensitive two pore-domain K^+ channels between the lumbar splanchnic and pelvic nerve innervations of mouse urinary bladder and colon," *Neuroscience*, vol. 186, pp. 179–187, 2011.

[139] S. M. Brierley, R. C. W. Jones III, G. F. Gebhart, and L. A. Blackshaw, "Splanchnic and pelvic mechanosensory afferents signal different qualities of colonic stimuli in mice," *Gastroenterology*, vol. 127, no. 1, pp. 166–178, 2004.

[140] S. M. Brierley, R. Carter, W. Jones III et al., "Differential chemosensory function and receptor expression of splanchnic and pelvic colonic afferents in mice," *Journal of Physiology*, vol. 567, no. 1, pp. 267–281, 2005.

[141] E. K. A. Corbett, J. K. Sinfield, P. N. McWilliam, J. Deuchars, and T. F. C. Batten, "Differential expression of vesicular glutamate transporters by vagal afferent terminals in rat nucleus of the solitary tract: projections from the heart preferentially express vesicular glutamate transporter 1," *Neuroscience*, vol. 135, no. 1, pp. 133–145, 2005.

[142] L. H. Lin and W. T. Talman, "Vesicular glutamate transporters and neuronal nitric oxide synthase colocalize in aortic depressor afferent neurons," *Journal of Chemical Neuroanatomy*, vol. 32, no. 1, pp. 54–64, 2006.

[143] A. Vandenbeuch, M. Tizzano, C. B. Anderson, L. M. Stone, D. Goldberg, and S. C. Kinnamon, "Evidence for a role of glutamate as an efferent transmitter in taste buds," *BMC Neuroscience*, vol. 11, article 77, 2010.

[144] J. P. Lund, S. Sadeghi, T. Athanassiadis et al., "Assessment of the potential role of muscle spindle mechanoreceptor afferents in chronic muscle pain in the rat masseter muscle," *PloS ONE*, vol. 5, no. 6, Article ID e11131, 2010.

[145] I. Brouns, I. Pintelon, J. van Genechten, I. de Proost, J.-P. Timmermans, and D. Adriaensen, "Vesicular glutamate transporter 2 is expressed in different nerve fibre populations that selectively contact pulmonary neuroepithelial bodies," *Histochemistry and Cell Biology*, vol. 121, no. 1, pp. 1–12, 2004.

[146] M. Ludwig, *Dendritic Neurotransmitter Release*, Springer, New York, NY, USA, 2004.

[147] Y. M. Ulrich-Lai, C. M. Flores, C. A. Harding-Rose, H. E. Goodis, and K. M. Hargreaves, "Capsaicin-evoked release of immunoreactive calcitonin gene-related peptide from rat trigeminal ganglion: Evidence for intraganglionic neurotransmission," *Pain*, vol. 91, no. 3, pp. 219–226, 2001.

[148] S. Thalakoti, V. V. Patil, S. Damodaram et al., "Neuron-glia signaling in trigeminal ganglion: implications for migraine pathology," *Headache*, vol. 47, no. 7, pp. 1008–1023, 2007.

[149] X. Zhang, Y. Chen, C. Wang, and L.-Y. M. Huang, "Neuronal somatic ATP release triggers neuron-satellite glial cell communication in dorsal root ganglia," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 23, pp. 9864–9869, 2007.

[150] J. M. Bráz, L. Ackerman, and A. I. Basbaum, "Sciatic nerve transection triggers release and intercellular transfer of a genetically expressed macromolecular tracer in dorsal root ganglia," *Journal of Comparative Neurology*, vol. 519, no. 13, pp. 2648–2657, 2011.

[151] B. Mackenzie, A. C. Illing, M. E. K. Morris, H. Varoqui, and J. D. Erickson, "Analysis of a vesicular glutamate transporter (VGLUT2) supports a cell-leakage mode in addition to vesicular packaging," *Neurochemical Research*, vol. 33, no. 2, pp. 238–247, 2008.

[152] K. E. Miller, V. D. Douglas, and T. Kaneko, "Glutaminase immunoreactive neurons in the rat dorsal root ganglion contain calcitonin gene-related peptide (CGRP)," *Neuroscience Letters*, vol. 160, no. 1, pp. 113–116, 1993.

[153] S. de Biasi and A. Rustioni, "Glutamate and substance P coexist in primary afferent terminals in the superficial laminae of spinal cord," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 85, no. 20, pp. 7820–7824, 1988.

[154] S. E. J. Hwang, A. Burette, A. Rustioni, and J. G. Valtschanoff, "Vanilloid receptor VR1-positive primary afferents are glutamatergic and contact spinal neurons that co-express neurokinin receptor NK1 and glutamate receptors," *Journal of Neurocytology*, vol. 33, no. 3, pp. 321–329, 2004.

[155] M. C. Lagerström, K. Rogoz, B. Abrahamsen et al., "VGLUT2-dependent sensory neurons in the TRPV1 population regulate pain and itch," *Neuron*, vol. 68, no. 3, pp. 529–542, 2010.

[156] T. Kaneko and F. Fujiyama, "Complementary distribution of vesicular glutamate transporters in the central nervous system," *Neuroscience Research*, vol. 42, no. 4, pp. 243–250, 2002.

[157] J.-L. Boulland, T. Qureshi, R. P. Seal et al., "Expression of the vesicular glutamate transporters during development indicates the widespread corelease of multiple neurotransmitters," *Journal of Comparative Neurology*, vol. 480, no. 3, pp. 264–280, 2004.

[158] R. T. Fremeau Jr., K. Kam, Y. Qureshi et al., "Vesicular glutamate transporters 1 and 2 target to functionally distinct synaptic release sites," *Science*, vol. 304, no. 5678, pp. 1815–1819, 2004.

[159] R. T. Fremeau Jr., S. Voglmaier, R. P. Seal, and R. H. Edwards, "VGLUTs define subsets of excitatory neurons and suggest novel roles for glutamate," *Trends in Neurosciences*, vol. 27, no. 2, pp. 98–103, 2004.

[160] J. S. Dittman and W. G. Regehr, "Calcium dependence and recovery kinetics of presynaptic depression at the climbing fiber to Purkinje cell synapse," *The Journal of Neuroscience*, vol. 18, no. 16, pp. 6147–6162, 1998.

[161] M. Raab and W. L. Neuhuber, "Vesicular glutamate transporter 2 immunoreactivity in putative vagal mechanosensor terminals of mouse and rat esophagus: Indication of a local effector function?" *Cell and Tissue Research*, vol. 312, no. 2, pp. 141–148, 2003.

[162] M. Raab and W. L. Neuhuber, "Number and distribution of intraganglionic laminar endings in the mouse esophagus as demonstrated with two different immunohistochemical markers," *Journal of Histochemistry and Cytochemistry*, vol. 53, no. 8, pp. 1023–1031, 2005.

[163] V. P. Zagorodnyuk, B. N. Chen, M. Costa, and S. J. H. Brookes, "Mechanotransduction by intraganglionic laminar endings of vagal tension receptors in the guinea-pig oesophagus," *Journal of Physiology*, vol. 553, no. 2, pp. 575–587, 2003.

[164] P. Ewald, W. L. Neuhuber, and M. Raab, "Vesicular glutamate transporter 1 immunoreactivity in extrinsic and intrinsic innervation of the rat esophagus," *Histochemistry and Cell Biology*, vol. 125, no. 4, pp. 377–395, 2006.

[165] T. Kraus, W. L. Neuhuber, and M. Raab, "Distribution of vesicular glutamate transporter 1 (VGLUT1) in the mouse esophagus," *Cell and Tissue Research*, vol. 329, no. 2, pp. 205–219, 2007.

[166] C. Olsson, M. Costa, and S. J. H. Brookes, "Neurochemical characterization of extrinsic innervation of the guinea pig rectum," *Journal of Comparative Neurology*, vol. 470, no. 4, pp. 357–371, 2004.

[167] N. J. Spencer, A. Kerrin, C. A. Singer, G. W. Hennig, W. T. Gerthoffer, and O. McDonnell, "Identification of capsaicin-sensitive rectal mechanoreceptors activated by rectal distension in mice," *Neuroscience*, vol. 153, no. 2, pp. 518–534, 2008.

[168] M.-T. Liu, J. D. Rothstein, M. D. Gershon, and A. L. Kirchgessner, "Glutamatergic enteric neurons," *The Journal of Neuroscience*, vol. 17, no. 12, pp. 4764–4784, 1997.

[169] I. S. Hitchcock, P. G. Genever, and P. M. B. Cahusac, "Essential components for a glutamatergic synapse between Merkel cell and nerve terminal in rats," *Neuroscience Letters*, vol. 362, no. 3, pp. 196–199, 2004.

[170] V. A. Botchkarev, S. Eichmuller, O. Johansson, and R. Paus, "Hair cycle-dependent plasticity of skin and hair follicle innervation in normal murine skin," *Journal of Comparative Neurology*, vol. 386, pp. 379–395, 1997.

[171] M. G. Nunzi, A. Pisarek, and E. Mugnaini, "Merkel cells, corpuscular nerve endings and free nerve endings in the mouse palatine mucosa express three subtypes of vesicular glutamate transporters," *Journal of Neurocytology*, vol. 33, pp. 359–376, 2004.

[172] W. C. de Groat and N. Yoshimura, "Afferent nerve regulation of bladder function in health and disease," *Handbook of Experimental Pharmacology*, vol. 194, pp. 91–138, 2009.

[173] H.-R. Berthoud, L. M. Patterson, F. Neumann, and W. L. Neuhuber, "Distribution and structure of vagal afferent intraganglionic laminar endings (IGLEs) in the rat gastrointestinal tract," *Anatomy and Embryology*, vol. 195, no. 2, pp. 183–191, 1997.

[174] S. P. Schneider and E. R. Perl, "Comparison of primary afferent and glutamate excitation of neurons in the mammalian spinal dorsal horn," *The Journal of Neuroscience*, vol. 8, no. 6, pp. 2062–2073, 1988.

[175] D. Bleakman, A. Alt, and E. S. Nisenbaum, "Glutamate receptors and pain," *Seminars in Cell and Developmental Biology*, vol. 17, no. 5, pp. 592–604, 2006.

[176] A. J. Todd and A. Ribeiro-da-Silva, "Molecular architecture of the dorsal horn," in *The Neurobiology of Pain*, pp. 65–94, Oxford University Press, Oxford, UK, 2005.

[177] S. P. Schneider and T. M. Walker, "Morphology and electrophysiological properties of hamster spinal dorsal horn neurons that express VGLUT2 and enkephalin," *Journal of Comparative Neurology*, vol. 501, no. 5, pp. 790–809, 2007.

[178] B. A. Du, S. S. Shakya, B. A. Bannatyne, S. M. Jalicy, S. Linnen, and D. J. Maxwell, "Neurotransmitter phenotypes of descending systems in the rat lumbar spinal cord," *Neuroscience*, vol. 227, pp. 67–79, 2012.

[179] S. Persson, J.-L. Boulland, M. Aspling et al., "Distribution of vesicular glutamate transporters 1 and 2 in the rat spinal cord, with a note on the spinocervical tract," *Journal of Comparative Neurology*, vol. 497, no. 5, pp. 683–701, 2006.

[180] S. A. Gebre, S. L. Reeber, and R. V. Sillitoe, "Parasagittal compartmentation of cerebellar mossy fibers as revealed by the patterned expression of vesicular glutamate transporters VGLUT1 and VGLUT2," *Brain Structure and Function*, vol. 217, pp. 165–180, 2011.

[181] R. V. Sillitoe, M. W. Vogel, and A. L. Joyner, "Engrailed homeobox genes regulate establishment of the cerebellar afferent circuit map," *The Journal of Neuroscience*, vol. 30, no. 30, pp. 10015–10024, 2010.

[182] D. J. Maxwell, M. D. Belle, O. Cheunsuang, A. Stewart, and R. Morris, "Morphology of inhibitory and excitatory interneurons in superficial laminae of the rat dorsal horn," *Journal of Physiology*, vol. 584, no. 2, pp. 521–533, 2007.

[183] T. Yasaka, S. Y. X. Tiong, D. I. Hughes, J. S. Riddell, and A. J. Todd, "Populations of inhibitory and excitatory interneurons in lamina II of the adult rat spinal dorsal horn revealed by a combined electrophysiological and anatomical approach," *Pain*, vol. 151, no. 2, pp. 475–488, 2010.

[184] A. Graziano, X.-B. Liu, K. D. Murray, and E. G. Jones, "Vesicular glutamate transporters define two sets of glutamatergic afferents to the somatosensory thalamus and two thalamocortical projections in the mouse," *Journal of Comparative Neurology*, vol. 507, no. 2, pp. 1258–1276, 2008.

[185] B. Meister, U. Arvidsson, X. Zhang, G. Jacobsson, M. J. Villar, and T. Hökfelt, "Glutamate transporter mRNA and glutamate-like immunoreactivity in spinal motoneurones," *NeuroReport*, vol. 5, no. 3, pp. 337–340, 1993.

[186] P. Lachamp, M. Crest, and J.-P. Kessler, "Vesicular glutamate transporters type 1 and 2 expression in axon terminals of the rat nucleus of the solitary tract," *Neuroscience*, vol. 137, no. 1, pp. 73–81, 2006.

[187] K. Kullander, S. J. B. Butt, J. M. Lebret et al., "Role of EphA4 and EphrinB3 in local neuronal circuits that control walking," *Science*, vol. 299, no. 5614, pp. 1889–1892, 2003.

[188] M. D. Mann, "Clarke's column and the dorsal spinocerebellar tract: a review," *Brain, Behavior and Evolution*, vol. 7, no. 1, pp. 34–83, 1973.

[189] O. Waerhaug and O. P. Ottersen, "Demonstration of glutamate-like immunoreactivity at rat neuromuscular junctions by quantitative electron microscopic immunocytochemistry," *Anatomy and Embryology*, vol. 188, no. 5, pp. 501–513, 1993.

[190] P. R. Brumovsky, T. J. Shi, H. Matsuda, J. Kopp, M. J. Villar, and T. Hökfelt, "NPY Y1 receptors are present in axonal processes of DRG neurons," *Experimental Neurology*, vol. 174, no. 1, pp. 1–10, 2002.

[191] X.-L. Gu and L.-C. Yu, "The colocalization of CGRP receptor and AMPA receptor in the spinal dorsal horn neuron of rat: a morphological and electrophysiological study," *Neuroscience Letters*, vol. 414, no. 3, pp. 237–241, 2007.

[192] G. G. Nagy, M. Al-Ayyan, D. Andrew, M. Fukaya, M. Watanabe, and A. J. Todd, "Widespread expression of the AMPA receptor GluR2 subunit at glutamatergic synapses in the rat spinal cord and phosphorylation of GluR1 in response to noxious stimulation revealed with an antigen-unmasking method," *The Journal of Neuroscience*, vol. 24, no. 25, pp. 5766–5777, 2004.

[193] J. M. Lundberg, T. Hökfelt, A. Anggard et al., "Peripheral peptide neurons: distribution, axonal transport, and some

aspects on possible function," in *Neural Peptides and Neuronal Communication*, E. Costa and M. Trabucchi, Eds., pp. 25–36, Raven Press, New York, NY, USA, 1980.

[194] M. R. Matthews and A. C. Cuello, "The origin and possible significance of substance P immunoreactive networks in the prevertebral ganglia and related structures in the guinea-pig," *Philosophical Transactions of the Royal Society of London B*, vol. 306, no. 1128, pp. 247–276, 1984.

[195] J. Aïoun and O. Rampin, "Anatomical evidence for glutamatergic transmission in primary sensory neurons and onto postganglionic neurons controlling penile erection in rats: an ultrastructural study with neuronal tracing and immunocytochemistry," *Cell and Tissue Research*, vol. 323, no. 3, pp. 359–375, 2006.

[196] G. B. Luckensmeyer and J. R. Keast, "Immunohistochemical characterisation of viscerofugal neurons projecting to the inferior mesenteric and major pelvic ganglia in the male rat," *Journal of the Autonomic Nervous System*, vol. 61, no. 1, pp. 6–16, 1996.

[197] T. J. Hibberd, V. P. Zagorodnyuk, N. J. Spencer, and S. J. Brookes, "Identification and mechanosensitivity of viscerofugal neurons," *Neuroscience*, vol. 225, pp. 118–129, 2012.

[198] S. M. Miller and J. H. Szurszewski, "Relationship between colonic motility and cholinergic mechanosensory afferent synaptic input to mouse superior mesenteric ganglion," *Neurogastroenterology and Motility*, vol. 14, no. 4, pp. 339–348, 2002.

[199] A. L. Kirchgessner, M.-T. Liu, and F. Alcantara, "Excitotoxicity in the enteric nervous system," *The Journal of Neuroscience*, vol. 17, no. 22, pp. 8804–8816, 1997.

[200] L. H. Tsai, W. Tsai, and J.-Y. Wu, "Effect of L-glutamic acid on acid secretion and immunohistochemical localization of glutamatergic neurons in the rat stomach," *The Journal of Neuroscience Research*, vol. 38, no. 2, pp. 188–195, 1994.

[201] C. Giaroni, E. Zanetti, A. M. Chiaravalli et al., "Evidence for a glutamatergic modulation of the cholinergic function in the human enteric nervous system via NMDA receptors," *European Journal of Pharmacology*, vol. 476, no. 1-2, pp. 63–69, 2003.

[202] J. W. Wiley, Y. Lu, and C. Owyang, "Evidence for a glutamatergic neural pathway in the myenteric plexus," *The American Journal of Physiology: Gastrointestinal and Liver Physiology*, vol. 261, no. 4, pp. G693–G700, 1991.

[203] M. Šinský and J. Donnerer, "Evidence for a neurotransmitter role of glutamate in guinea pig myenteric plexus neurons," *Neuroscience Letters*, vol. 258, no. 2, pp. 109–112, 1998.

[204] A. L. Kirchgessner, "Glutamate in the enteric nervous system," *Current Opinion in Pharmacology*, vol. 1, no. 6, pp. 591–596, 2001.

[205] N. Linke, N. Bódi, B. E. Resch, É. Fekete, and M. Bagyánszki, "Developmental pattern of three vesicular glutamate transporters in the myenteric plexus of the human fetal small intestine," *Histology and Histopathology*, vol. 23, no. 7–9, pp. 979–986, 2008.

[206] X. Zhang, D. A. Dagerlind, L. Bao, R. R. Ji, J. M. Lundberg, and T. Hökfelt, "Increased expression of galanin in the rat superior cervical ganglion after pre- and postganglionic nerve lesions," *Experimental Neurology*, vol. 127, no. 1, pp. 9–22, 1994.

[207] M. Costigan, K. Befort, L. Karchewski et al., "Replicate high-density rat genome oligonucleotide microarrays reveal hundreds of regulated genes in the dorsal root ganglion after peripheral nerve injury," *BMC Neuroscience*, vol. 3, article 16, 2002.

[208] H.-S. Xiao, Q.-H. Huang, F.-X. Zhang et al., "Identification of gene expression profile of dorsal root ganglion in the rat peripheral axotomy model of neuropathic pain," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 12, pp. 8360–8365, 2002.

[209] X. Navarro, "Chapter 27: neural plasticity after nerve injury and regeneration," *International Review of Neurobiology*, vol. 87, pp. 483–505, 2009.

[210] X. Zhang, Z.-Q. Xu, T.-J. Shi et al., "Regulation of expression of galanin and galanin receptors in dorsal root ganglia and spinal cord after axotomy and inflammation," *Annals of the New York Academy of Sciences*, vol. 863, pp. 402–413, 1998.

[211] K. Ren, S. I. Novikova, F. He, R. Dubner, and M. S. Lidow, "Neonatal local noxious insult affects gene expression in the spinal dorsal horn of adult rats," *Molecular Pain*, vol. 1, article 27, 2005.

[212] J. A. Christianson, K. Bielefeldt, C. Altier et al., "Development, plasticity and modulation of visceral afferents," *Brain Research Reviews*, vol. 60, no. 1, pp. 171–186, 2009.

[213] W. M. Al-Ghoul, G. L. Volsi, R. J. Weinberg, and A. Rustioni, "Glutamate immunocytochemistry in the dorsal horn after injury or stimulation of the sciatic nerve of rats," *Brain Research Bulletin*, vol. 30, no. 3-4, pp. 453–459, 1993.

[214] D. I. Hughes, E. Polgár, S. A. S. Shehab, and A. J. Todd, "Peripheral axotomy induces depletion of the vesicular glutamate transporter VGLUT1 in central terminals of myelinated afferent fibres in the rat spinal cord," *Brain Research*, vol. 1017, no. 1-2, pp. 69–76, 2004.

[215] P. R. Brumovsky, E. Bergman, H.-X. Liu, T. Hökfelt, and M. J. Villar, "Effect of a graded single constriction of the rat sciatic nerve on pain behavior and expression of immunoreactive NPY and NPY Y1 receptor in DRG neurons and spinal cord," *Brain Research*, vol. 1006, no. 1, pp. 87–99, 2004.

[216] F. A. Chaudhry, J.-L. Boulland, M. Jenstad, M. K. L. Bredahl, and R. H. Edwards, "Pharmacology of neurotransmitter transport into secretory vesicles," *Handbook of Experimental Pharmacology*, no. 184, pp. 77–106, 2008.

[217] D. E. Featherstone, "Intercellular glutamate signaling in the nervous system and beyond," *ACS Chemical Neuroscience*, vol. 1, no. 1, pp. 4–12, 2010.

[218] B. Lindh, M. Risling, S. Remahl, L. Terenius, and T. Hökfelt, "Peptide-immunoreactive neurons and nerve fibres in lumbosacral sympathetic ganglia: Selective elimination of a pathway-specific expression of immunoreactivities following sciatic nerve resection in kittens," *Neuroscience*, vol. 55, no. 2, pp. 545–562, 1993.

[219] T. B. Cheah and L. B. Geffen, "Effects of axonal injury on norepinephrine, tyrosine hydroxylase and monoamine oxidase levels in sympathetic ganglia," *Journal of Neurobiology*, vol. 4, no. 5, pp. 443–452, 1973.

[220] Y. Sun and R. E. Zigmond, "Involvement of leukemia inhibitory factor in the increases in galanin and vasoactive intestinal peptide mRNA and the decreases in neuropeptide Y and tyrosine hydroxylase mRNA in sympathetic neurons after axotomy," *Journal of Neurochemistry*, vol. 67, no. 4, pp. 1751–1760, 1996.

[221] M. S. Rao, Y. Sun, U. Vaidyanathan, S. C. Landis, and R. E. Zigmond, "Regulation of substance P is similar to that of vasoactive intestinal peptide after axotomy or explantation of the rat superior cervical ganglion," *Journal of Neurobiology*, vol. 24, no. 5, pp. 571–580, 1993.

[222] C. Skobowiat, J. Calka, and M. Majewski, "Axotomy induced changes in neuronal plasticity of sympathetic chain ganglia (SChG) neurons supplying descending colon in the pig," *Experimental and Molecular Pathology*, vol. 90, no. 1, pp. 13–18, 2011.

[223] D. Sulzer, M. P. Joyce, L. Lin et al., "Dopamine neurons make glutamatergic synapses in vitro," *The Journal of Neuroscience*, vol. 18, pp. 4588–4602, 1998.

[224] A. Kawasaki, K. Hoshi, M. Kawano, H. Nogami, H. Yoshikawa, and S. Hisano, "Up-regulation of VGLUT2 expression in hypothalamic-neurohypophysial neurons of the rat following osmotic challenge," *European Journal of Neuroscience*, vol. 22, no. 3, pp. 672–680, 2005.

[225] T. Yamaguchi, W. Sheen, and M. Morales, "Glutamatergic neurons are present in the rat ventral tegmental area," *European Journal of Neuroscience*, vol. 25, no. 1, pp. 106–118, 2007.

[226] R. L. Stornetta, C. P. Sevigny, A. M. Schreihofer, D. L. Rosin, and P. G. Guyenet, "Vesicular glutamate transporter DNPI/VGLUT2 is expressed by both C1 adrenergic and nonaminergic presympathetic vasomotor neurons of the rat medulla," *Journal of Comparative Neurology*, vol. 444, no. 3, pp. 207–220, 2002.

[227] R. L. Stornetta, C. P. Sevigny, and P. G. Guyenet, "Vesicular glutamate transporter DNPI/VGLUT2 mRNA is present in C1 and several other groups of brainstem catecholaminergic neurons," *Journal of Comparative Neurology*, vol. 444, no. 3, pp. 191–206, 2002.

[228] M. Kawano, A. Kawasaki, H. Sakata-Haga et al., "Particular subpopulations of midbrain and hypothalamic dopamine neurons express vesicular glutamate transporter 2 in the rat brain," *Journal of Comparative Neurology*, vol. 498, no. 5, pp. 581–592, 2006.

[229] U. Ernsberger and H. Rohrer, "Development of the cholinergic neurotransmitter phenotype in postganglionic sympathetic neurons," *Cell and Tissue Research*, vol. 297, no. 3, pp. 339–361, 1999.

[230] S. M. Wojcik, J. S. Rhee, E. Herzog et al., "An essential role for vesicular glutamate transporter 1 (VGLUT1) in postnatal development and control of quantal size," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 18, pp. 7158–7163, 2004.

[231] N. R. Wilson, J. Kang, E. V. Hueske et al., "Presynaptic regulation of quantal size by the vesicular glutamate transporter VGLUT1," *The Journal of Neuroscience*, vol. 25, no. 26, pp. 6221–6234, 2005.

[232] R. W. Daniels, C. A. Collins, K. Chen, M. V. Gelfand, D. E. Featherstone, and A. DiAntonio, "A single vesicular glutamate transporter is sufficient to fill a synaptic vesicle," *Neuron*, vol. 49, no. 1, pp. 11–16, 2006.

[233] S. E. Kapadia and C. C. LaMotte, "Deafferentation-induced alterations in the rat dorsal horn: I. Comparison of peripheral nerve injury vs. rhizotomy effects on presynaptic, postsynaptic and glial processes," *Journal of Comparative Neurology*, vol. 266, no. 2, pp. 183–197, 1987.

[234] X. Zhang, A. J. Bean, Z. Wiesenfeld-Hallin, X.-J. Xu, and T. Hökfelt, "Ultrastructural studies on peptides in the dorsal horn of the rat spinal cord - III. Effects of peripheral axotomy with special reference to galanin," *Neuroscience*, vol. 64, no. 4, pp. 893–915, 1995.

[235] H. Fei, A. Grygoruk, E. S. Brooks, A. Chen, and D. E. Krantz, "Trafficking of vesicular neurotransmitter transporters," *Traffic*, vol. 9, no. 9, pp. 1425–1436, 2008.

[236] M. S. Santos, H. Li, and S. M. Voglmaier, "Synaptic vesicle protein trafficking at the glutamate synapse," *Neuroscience*, vol. 158, no. 1, pp. 189–203, 2009.

[237] S. Schenck, S. M. Wojcik, N. Brose, and S. Takamori, "A chloride conductance in VGLUT1 underlies maximal glutamate loading into synaptic vesicles," *Nature Neuroscience*, vol. 12, no. 2, pp. 156–162, 2009.

[238] S. Karunanithi, L. Marin, K. Wong, and H. L. Atwood, "Quantal size and variation determined by vesicle size in normal and mutant Drosophila glutamatergic synapses," *The Journal of Neuroscience*, vol. 22, no. 23, pp. 10267–10276, 2002.

[239] K. E. Miller, J. C. Balbas, R. L. Benton et al., "Glutaminase immunoreactivity and enzyme activity is increased in the rat dorsal root ganglion following peripheral inflammation," *Pain Research and Treatment*, vol. 2012, Article ID 414697, 9 pages, 2012.

[240] S. Leo, D. Moechars, Z. Callaerts-Vegh, R. D'Hooge, and T. Meert, "Impairment of VGLUT2 but not VGLUT1 signaling reduces neuropathy-induced hypersensitivity," *European Journal of Pain*, vol. 13, no. 10, pp. 1008–1017, 2009.

[241] D. Moechars, M. C. Weston, S. Leo et al., "Vesicular glutamate transporter VGLUT2 expression levels control quantal size and neuropathic pain," *The Journal of Neuroscience*, vol. 26, no. 46, pp. 12055–12066, 2006.

[242] G. Grant and B. Robertsson, "Primary afferent projections to the spinal cord," in *The Rat Nervous System*, G. Paxinos, Ed., pp. 112–120, Elsevier Academic Press, London, UK, 2004.

[243] M. C. Lagerström, K. Rogoz, B. Abrahamsen et al., "A sensory subpopulation depends on vesicular glutamate transporter 2 for mechanical pain, and together with substance P, inflammatory pain," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 14, pp. 5789–5794, 2011.

[244] Y. Liu, O. Abdel Samad, L. Zhang et al., "VGLUT2-dependent glutamate release from nociceptors is required to sense pain and suppress itch," *Neuron*, vol. 68, no. 3, pp. 543–556, 2010.

[245] F. Lallemend and P. Ernfors, "Molecular interactions underlying the specification of sensory neurons," *Trends in Neurosciences*, vol. 35, no. 6, pp. 373–381, 2012.

[246] J. deGroot, S. Zhou, and S. M. Carlton, "Peripheral glutamate release in the hindpaw following low and high intensity sciatic stimulation," *NeuroReport*, vol. 11, no. 3, pp. 497–502, 2000.

[247] S. M. Carlton, G. L. Hargett, and R. E. Coggeshall, "Localization and activation of glutamate receptors in unmyelinated axons of rat glabrous skin," *Neuroscience Letters*, vol. 197, no. 1, pp. 25–28, 1995.

[248] D. L. Jackson, C. B. Graff, J. D. Richardson, and K. M. Hargreaves, "Glutamate participates in the peripheral modulation of thermal hyperalgesia in rats," *European Journal of Pharmacology*, vol. 284, no. 3, pp. 321–325, 1995.

[249] Y.-L. Tian, Y. Guo, D.-Y. Cao, Q. Zhang, H.-S. Wang, and Y. Zhao, "Local application of morphine suppresses glutamate-evoked activities of C and Aδ afferent fibers in rat hairy skin," *Brain Research*, vol. 1059, no. 1, pp. 28–34, 2005.

[250] K. Sato, H. Kiyama, H. T. Park, and M. Tohyama, "AMPA, KA and NMDA receptors are expressed in the rat DRG neurones," *NeuroReport*, vol. 4, no. 11, pp. 1263–1265, 1993.

[251] M. Tachibana, R. J. Wenthold, H. Morioka, and R. S. Petralia, "Light and electron microscopic immunocytochemical localization of AMPA- selective glutamate receptors in the rat spinal cord," *Journal of Comparative Neurology*, vol. 344, no. 3, pp. 431–454, 1994.

[252] H. Ohishi, S. Nomura, Y.-Q. Ding et al., "Presynaptic localization of a metabotropic glutamate receptor, mGluR7, in the primary afferent neurons: an immunohistochemical study in the rat," *Neuroscience Letters*, vol. 202, no. 1-2, pp. 85–88, 1995.

[253] S. M. Carlton, "Peripheral excitatory amino acids," *Current Opinion in Pharmacology*, vol. 1, no. 1, pp. 52–56, 2001.

[254] S. Zhou, S. Komak, J. Du, and S. M. Carlton, "Metabotropic glutamate 1α receptors on peripheral primary afferent fibers: their role in nociception," *Brain Research*, vol. 913, no. 1, pp. 18–26, 2001.

[255] V. V. Chaban, "Visceral sensory neurons that innervate both uterus and colon express nociceptive TRPV1 and P2X3 receptors in rats," *Ethnicity and Disease*, vol. 18, no. 2, pp. S2–S4, 2008.

[256] T. Sugiura, K. Bielefeldt, and G. F. Gebhart, "Mouse colon sensory neurons detect extracellular acidosis via TRPV1," *The American Journal of Physiology: Cell Physiology*, vol. 292, no. 5, pp. C1768–C1774, 2007.

[257] K. Wirkner, B. Sperlagh, and P. Illes, "P_2X_3 receptor involvement in pain states," *Molecular Neurobiology*, vol. 36, no. 2, pp. 165–183, 2007.

[258] M. Shinoda, B. Feng, and G. F. Gebhart, "Peripheral and central P_2X_3 receptor contributions to colon mechanosensitivity and hypersensitivity in the mouse," *Gastroenterology*, vol. 137, no. 6, pp. 2096–2104, 2009.

[259] G. Burnstock, "Purinergic mechanosensory transduction and visceral pain," *Molecular Pain*, vol. 5, p. 69, 2009.

[260] J. M. A. Laird, V. Souslova, J. N. Wood, and F. Cervero, "Deficits in visceral pain and referred hyperalgesia in Nav1.8 (SNS/PN3)-null mice," *The Journal of Neuroscience*, vol. 22, no. 19, pp. 8352–8356, 2002.

[261] W. Jänig and R. Baron, "Complex regional pain syndrome: mystery explained?" *Lancet Neurology*, vol. 2, no. 11, pp. 687–697, 2003.

[262] G. F. Gibbs, P. D. Drummond, P. M. Finch, and J. K. Phillips, "Unravelling the pathophysiology of complex regional pain syndrome: focus on sympathetically maintained pain," *Clinical and Experimental Pharmacology and Physiology*, vol. 35, no. 7, pp. 717–724, 2008.

[263] R. Shigemoto, H. Ohishi, S. Nakanishi, and N. Mizuno, "Expression of the mRNA for the rat NMDA receptor (NMDAR1) in the sensory and autonomic ganglion neurons," *Neuroscience Letters*, vol. 144, no. 1-2, pp. 229–232, 1992.

[264] P. J. Kammermeier and S. R. Ikeda, "Metabotropic glutamate receptor expression in the rat superior cervical ganglion," *Neuroscience Letters*, vol. 330, no. 3, pp. 260–264, 2002.

[265] H. Kiyama, K. Sato, T. Kuba, and M. Tohyama, "Sympathetic and parasympathetic ganglia express non-NMDA type glutamate receptors: distinct receptor subunit composition in the principle and SIF cells," *Molecular Brain Research*, vol. 19, no. 4, pp. 345–348, 1993.

[266] S. M. Carlton, K. Chung, Z. Ding, and R. E. Coggeshall, "Glutamate receptors on postganglionic sympathetic axons," *Neuroscience*, vol. 83, no. 2, pp. 601–605, 1998.

[267] J. A. McRoberts, S. V. Coutinho, J. C. G. Marvizón et al., "Role of peripheral N-methyl-D-aspartate (NMDA) receptors in visceral nociception in rats," *Gastroenterology*, vol. 120, no. 7, pp. 1737–1748, 2001.

[268] R. L. Young, A. J. Page, T. A. O'Donnell, N. J. Cooper, and L. A. Blackshaw, "Peripheral versus central modulation of gastric vagal pathways by metabotropic glutamate receptor 5," *The American Journal of Physiology: Gastrointestinal and Liver Physiology*, vol. 292, no. 2, pp. G501–G511, 2007.

[269] L. A. Blackshaw, A. J. Page, and R. L. Young, "Metabotropic glutamate receptors as novel therapeutic targets on visceral sensory pathways," *Frontiers in Neuroscience*, vol. 5, p. 40, 2011.

[270] M. Julio-Pieper, R. M. O. 'Connor, T. G. Dinan, and J. F. Cryan, "Regulation of the brain-gut axis by group III metabotropic glutamate receptors," *European Journal of Pharmacology*, vol. 698, pp. 19–30, 2013.

[271] J. C. G. Marvizón, J. A. McRoberts, H. S. Ennes et al., "Two N-methyl-D-aspartate receptors in rat dorsal root ganglia with different subunit composition and localization," *Journal of Comparative Neurology*, vol. 446, no. 4, pp. 325–341, 2002.

[272] S. Lucifora, H. H. Willcockson, C.-R. Lu et al., "Presynaptic low- and high-affinity kainate receptors in nociceptive spinal afferents," *Pain*, vol. 120, no. 1-2, pp. 97–105, 2006.

[273] S. M. Carlton and G. L. Hargett, "Colocalization of metabotropic glutamate receptors in rat dorsal root ganglion cells," *Journal of Comparative Neurology*, vol. 501, no. 5, pp. 780–789, 2007.

[274] C. Goudet, V. Magnaghi, M. Landry, F. Nagy, R. W. Gereau IV, and J.-P. Pin, "Metabotropic receptors for glutamate and GABA in pain," *Brain Research Reviews*, vol. 60, no. 1, pp. 43–56, 2009.

[275] J. L. Rozas, "Metabotropic actions of kainate receptors in dorsal root ganglion cells," *Advances in Experimental Medicine and Biology*, vol. 717, pp. 69–80, 2011.

[276] H. Tsujino, E. Kondo, T. Fukuoka et al., "Activating transcription factor 3 (ATF3) induction by axotomy in sensory and motoneurons: a novel neuronal marker of nerve injury," *Molecular and Cellular Neurosciences*, vol. 15, no. 2, pp. 170–182, 2000.

[277] H. Hyatt Sachs, R. C. Schreiber, S. E. Shoemaker, A. Sabe, E. Reed, and R. E. Zigmond, "Activating transcription factor 3 induction in sympathetic neurons after axotomy: Response to decreased neurotrophin availability," *Neuroscience*, vol. 150, no. 4, pp. 887–897, 2007.

[278] R. Seijffers, C. D. Mills, and C. J. Woolf, "ATF3 increases the intrinsic growth state of DRG neurons to enhance peripheral nerve regeneration," *The Journal of Neuroscience*, vol. 27, no. 30, pp. 7911–7920, 2007.

[279] S. Honma, M. Kawano, S. Hayashi, H. Kawano, and S. Hisano, "Expression and immunohistochemical localization of vesicular glutamate transporter 2 in the migratory pathway from the rat olfactory placode," *European Journal of Neuroscience*, vol. 20, no. 4, pp. 923–936, 2004.

[280] N. Bérubé-Carrière, M. Riad, G. Dal Bo, D. Lévesque, L.-É. Trudeau, and L. Descarries, "The dual dopamine-glutamate phenotype of growing mesencephalic neurons regresses in mature rat brain," *Journal of Comparative Neurology*, vol. 517, no. 6, pp. 873–891, 2009.

[281] G. M. Fortin, M. J. Bourque, J. A. Mendez et al., "Glutamate corelease promotes growth and survival of midbrain dopamine neurons," *The Journal of Neuroscience*, vol. 32, pp. 17477–17491, 2012.

[282] M. P. Mattson, "Excitotoxic and excitoprotective mechanisms: abundant targets for the prevention and treatment of neurodegenerative disorders," *NeuroMolecular Medicine*, vol. 3, no. 2, pp. 65–94, 2003.

[283] R. Balazs, "Trophic effect of glutamate," *Current Topics in Medicinal Chemistry*, vol. 6, no. 10, pp. 961–968, 2006.

[284] S.-H. Woo, Y. Baba, A. M. Franco, E. A. Lumpkin, and D. M. Owens, "Excitatory glutamate is essential for development and maintenance of the piloneural mechanoreceptor," *Development*, vol. 139, no. 4, pp. 740–748, 2012.

[285] J. Williams, "How does a vesicle know it is full?" *Neuron*, vol. 18, no. 5, pp. 683–686, 1997.

[286] R. W. Daniels, C. A. Collins, M. V. Gelfand et al., "Increased expression of the Drosophila vesicular glutamate transporter leads to excess glutamate release and a compensatory decrease

in quantal content," *The Journal of Neuroscience*, vol. 24, no. 46, pp. 10466–10474, 2004.

[287] S. Roseth, E. M. Fykse, and F. Fonnum, "Uptake of L-glutamate into rat brain synaptic vesicles: effect of inhibitors that bind specifically to the glutamate transporter," *Journal of Neurochemistry*, vol. 65, no. 1, pp. 96–103, 1995.

[288] Z. He, L. Yan, Z. Yong, Z. Dong, H. Dong, and Z. Gong, "Chicago sky blue 6B, a vesicular glutamate transporters inhibitor, attenuates methamphetamine-induced hyperactivity and behavioral sensitization in mice, Behav," *Behavioural Brain Research*, vol. 239, pp. 172–176, 2013.

[289] A. Beirith, A. R. S. Santos, and J. B. Calixto, "Mechanisms underlying the nociception and paw oedema caused by injection of glutamate into the mouse paw," *Brain Research*, vol. 924, no. 2, pp. 219–228, 2002.

[290] N. Pietrancosta, A. Kessler, F.-C. Favre-Besse et al., "Rose Bengal analogs and vesicular glutamate transporters (VGLUTs)," *Bioorganic and Medicinal Chemistry*, vol. 18, no. 18, pp. 6922–6933, 2010.

[291] H. Omote, T. Miyaji, N. Juge, and Y. Moriyama, "Vesicular neurotransmitter transporter: bioenergetics and regulation of glutamate transport," *Biochemistry*, vol. 50, no. 25, pp. 5558–5565, 2011.

[292] D. N. Ruskin and S. A. Masino, "The nervous system and metabolic dysregulation: emerging evidence converges on ketogenic diet therapy," *Frontiers in Neuroscience*, vol. 6, p. 33, 2012.

[293] D. N. Ruskin, M. Kawamura Jr., and S. A. Masino, "Reduced pain and inflammation in juvenile and adult rats fed a ketogenic diet," *PLoS ONE*, vol. 4, no. 12, Article ID e8349, 2009.

[294] T. M. Lund, Ø. Risa, U. Sonnewald, A. Schousboe, and H. S. Waagepetersen, "Availability of neurotransmitter glutamate is diminished when β-hydroxybutyrate replaces glucose in cultured neurons," *Journal of Neurochemistry*, vol. 110, no. 1, pp. 80–91, 2009.

[295] N. Juge, J. A. Gray, H. Omote et al., "Metabolic control of vesicular glutamate transport and release," *Neuron*, vol. 68, no. 1, pp. 99–112, 2010.

[296] S. O. Heyliger, C. B. Goodman, J. M. Ngong, and K. F. A. Soliman, "The analgesic effects of tryptophan and its metabolites in the rat," *Pharmacological Research*, vol. 38, no. 4, pp. 243–250, 1998.

[297] F. Fazio, L. Lionetto, G. Molinaro et al., "Cinnabarinic acid, an endogenous metabolite of the kynurenine pathway, activates type 4 metabotropic glutamate receptors," *Molecular Pharmacology*, vol. 81, no. 5, pp. 643–656, 2012.

[298] S. A. Neale, C. S. Copeland, V. N. Uebele, F. J. Thomson, and T. E. Salt, "Modulation of hippocampal synaptic transmission by the kynurenine pathway member xanthurenic acid and other VGLUT inhibitors," *Neuropsychopharmacology*, vol. 38, pp. 1060–1067, 2013.

[299] C. N. Carrigan, R. D. Bartlett, C. S. Esslinger et al., "Synthesis and in vitro pharmacology of substituted quinoline-2,4-dicarboxylic acids as inhibitors of vesicular glutamate transport," *Journal of Medicinal Chemistry*, vol. 45, no. 11, pp. 2260–2276, 2002.

[300] S. A. Patel, J. O. Nagy, E. D. Bolstad, J. M. Gerdes, and C. M. Thompson, "Tetrapeptide inhibitors of the glutamate vesicular transporter (VGLUT)," *Bioorganic and Medicinal Chemistry Letters*, vol. 17, no. 18, pp. 5125–5128, 2007.

[301] G. Sanacora, C. A. Zarate, J. H. Krystal, and H. K. Manji, "Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders," *Nature Reviews Drug Discovery*, vol. 7, no. 5, pp. 426–437, 2008.

[302] T. J. Coderre, N. Kumar, C. D. Lefebvre, and J. S. C. Yu, "A comparison of the glutamate release inhibition and anti-allodynic effects of gabapentin, lamotrigine, and riluzole in a model of neuropathic pain," *Journal of Neurochemistry*, vol. 100, no. 5, pp. 1289–1299, 2007.

[303] M. E. Fundytus, "Glutamate receptors and nociception: implications for the drug treatment of pain," *CNS Drugs*, vol. 15, no. 1, pp. 29–58, 2001.

[304] G.-A. Gaudreau and V. Plourde, "Involvement of N-methyl-D-aspartate (NMDA) receptors in a rat model of visceral hypersensitivity," *Behavioural Brain Research*, vol. 150, no. 1-2, pp. 185–189, 2004.

[305] J. Sawynok, "Topical and peripherally acting analgesics," *Pharmacological Reviews*, vol. 55, no. 1, pp. 1–20, 2003.

[306] S. M. Carlton, "Peripheral NMDA receptors revisited: hope floats," *Pain*, vol. 146, no. 1-2, pp. 1–2, 2009.

[307] E. M. Hoffman and K. E. Miller, "Peripheral inhibition of glutaminase reduces carrageenan-induced Fos expression in the superficial dorsal horn of the rat," *Neuroscience Letters*, vol. 472, no. 3, pp. 157–160, 2010.