Inhibitory neurotransmission regulates vagal efferent activity and gastric motility

Caitlin A McMenamin, R Alberto Travagli and Kirsteen N Browning

Department of Neural and Behavioral Sciences, Penn State College of Medicine, Hershey, PA 17033, USA
Corresponding author: Kirsteen N Browning. Email: knb13@psu.edu

Abstract

The gastrointestinal tract receives extrinsic innervation from both the sympathetic and parasympathetic nervous systems, which regulate and modulate the function of the intrinsic (enteric) nervous system. The stomach and upper gastrointestinal tract in particular are heavily influenced by the parasympathetic nervous system, supplied by the vagus nerve, and disruption of vagal sensory or motor functions results in disorganized motility patterns, disrupted receptive relaxation and accommodation, and delayed gastric emptying, amongst others. Studies from several laboratories have shown that the activity of vagal efferent motoneurons innervating the upper GI tract is inhibited tonically by GABAergic synaptic inputs from the adjacent nucleus tractus solitarius. Disruption of this influential central GABA input impacts vagal efferent output, hence gastric functions, significantly. The purpose of this review is to describe the development, physiology, and pathophysiology of this functionally dominant inhibitory synapse and its role in regulating vagally determined gastric functions.

Keywords: Inhibitory neurotransmission, GABAergic signaling, gastric motility, DMV, NTS, vagus

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Introduction

Appropriate gastrointestinal (GI) functions are critical for a variety of factors including nutrient absorption, satiety signaling, and energy homeostasis but also for drug absorption and delivery. GI dysfunctions (examples in Table 1 left) often accompany, and potentially exacerbate, other underlying diseases (examples in Table 1 right). Almost 45% of the US population report upper GI symptoms, yet treatments are often less than effective because of a lack of understanding of underlying pathophysiology and appropriate therapeutic interventions.[1–4]

While the GI tract possesses intrinsic neural plexuses that allow a significant degree of independent control over GI functions, the central nervous system (CNS) provides extrinsic neural inputs that regulate and modulate these functions. The esophagus and stomach, in particular, are more dependent upon extrinsic neural inputs; the sympathetic nervous system exerts predominately inhibitory effects upon GI smooth muscle and mucosal secretion while also regulating blood flow via vasoconstriction. The parasympathetic nervous system, in contrast, exerts both excitatory and inhibitory control over GI motility and tone and removal of this parasympathetic innervation results in disorganized and disrupted motility patterns that often induce nausea, vomiting, abdominal pain, and discomfort.[5]

The parasympathetic innervation to the GI tract is provided by the vagus nerve. Vagal sensory and motor circuits provide a pathway for communication between the GI tract and the CNS which allows the integration, modulation, and synchronization of digestive processes. The sensory (afferent) limb of this circuit has been studied more frequently, and its role in various pathologies including obesity, diabetes, gastritis, gastric ulcer and inflammation is better understood.[6–14] The aim of this review, however, is to examine the less well described motor (efferent) limb and, more importantly, the functionally dominant inhibitory synaptic input onto vagal efferent motoneurons, which ultimately determines vagally modulated gastric functions.

Vagal sensorimotor neurocircuits

The peripheral terminals of vagal afferents innervate all layers of the GI tract and their anatomy and morphology influence the modality of their response (chemical, osmotic, mechanical etc.[15]). The cell bodies of these afferents, located in the nodose ganglion, are pseudounipolar neurons whose central terminals have glutamatergic inputs onto the nucleus tractus solitarius (NTS) in the brainstem,[5,16,17] a primary site for neuronal integration. The NTS, for example, has reciprocal connections with the parabrachial nucleus, the hypothalamus, the periaqueductal gray area, and the central nucleus of the amygdala, and also receives inputs

Table 1 Examples of physiology and pathophysiology of vagally dependent visceral functions

Selected vagally dependent gastrointestinal functions	Selected diseases associated with altered vagally dependent functions
Swallowing	Dysphagia and achalasia
Gastric motility, and emptying	Nausea and vomiting
Gastric acid secretion	Delayed gastric emptying and gastroparesis
Receptive relaxation; gastric accommodation	Diabetic gastropathy (Type 1 and 2 diabetes)
Intestinal motility	Functional dyspepsia
Pancreatic exocrine and endocrine secretion	Early satiety
Satiation and regulation of food intake	Gastric ulcers
Cholinergic anti-inflammatory reflex	Acute pancreatitis
	Irritable bowel syndrome
	Neurological disorders such as Parkinson's disease

from the raphe, trigeminal, and vestibular nuclei, the area postrema, spinal cord, and prefrontal cortex.[5] In terms of vagal efferent control of the GI tract, the NTS relays this integrated signal to the adjacent dorsal motor nucleus of the vagus (DMV) using primarily GABAergic, glutamatergic, and catecholaminergic projections.[18–21]

Gastric motility is influenced heavily by the efferent (motor) output of DMV neurons. While DMV neurons are pacemakers, having a low intrinsic level of spontaneous action potential firing[21], their activity and excitability are regulated heavily by synaptic inputs, particularly inputs from the NTS. Retrograde tracing experiments suggest that, rather than being organized viscerotopically, the DMV is organized in rostro-caudal ''columns,'' each of which contains the motoneurons that innervate the subdiaphragmatic viscera through one of the five subdiaphragmatic vagal branches: anterior gastric, posterior gastric, hepatic, celiac, and accessory celiac.[22–24] In general, the medial and lateral portions of the DMV contain neurons innervating the more proximal and distal GI organs, respectively.[25] As preganglionic parasympathetic neurons, the overwhelming majority of DMV neurons are cholinergic.[26] They belong, however, to one of the two pathways; an excitatory, cholinergic or an inhibitory, non-adrenergic, non-cholinergic, (NANC) pathway, determined by the postganglionic neurons they synapse onto within the enteric nervous system. The excitatory cholinergic pathway releases acetylcholine which activates muscarinic receptors on GI smooth muscle to increase motility and tone, while the inhibitory NANC pathway releases either nitric oxide (NO) or vasoactive intestinal peptide (VIP) to decrease motility and tone.[27] A decrease in gastric motility can occur, therefore, by either inhibiting the excitatory cholinergic pathway or activating the inhibitory NANC pathway.

Gastroexcitatory neurons appear to be located in the more rostral and medial divisions of the DMV, while gastroinhibitory neurons appear to be located in more rostrolateral and caudomedial areas of the DMV.[28,29] The excitatory cholinergic pathway is active tonically; hence, the upper GI tract receives an ongoing level of excitatory activity. Previous studies have demonstrated, however, that microinjections of the non-selective ionotropic glutamatergic receptor antagonist, kynurenic acid, into the dorsal vagal complex (i.e., NTS, DMV and area postrema) had little to no effect on gastric motility, while microinjection of the $GABA_A$ receptor antagonist, bicuculline, caused large increases in gastric tone and motility. This suggests that the vagal efferent output of DMV neurons is suppressed by a tonic inhibitory GABAergic input. Disruption of this influential central GABA input, therefore, has a significant effect on vagal efferent output and gastric functions.[20,27,30]

GABAergic signaling in vagal neurocircuits

In mature DMV neurons, both phasic (synaptic) and tonic (extrasynaptic) GABA currents modulate the excitability and activity of vagal efferent motoneurons.[21,30,31] Phasic and tonic GABA-dependent currents are associated with the activation of receptors with different receptor subunit compositions and locations on the postsynaptic membrane. $GABA_A$ receptors are heteropentameric, consisting of two α, two β, and an additional δ or γ subunit. $GABA_A$ receptors containing the γ subunit, for example, are located predominately within the synapse and are associated with transient, phasic inhibition of neurons.[32] Phasic $GABA_A$ currents occur during synaptic transmission involving release of high concentrations of GABA which induces a short-term inhibitory postsynaptic current (IPSC) in the postsynaptic neuron. In contrast, $GABA_A$ receptors containing the δ subunit are frequently expressed peri- and extra-synaptically, and are associated with long lasting, slow, tonic inhibition of neurons.[32] Tonic $GABA_A$ currents occur in response to diffusion of GABA from the synaptic cleft; low extracellular GABA concentrations can activate extrasynaptic receptors which are responsible, in part, for narrowing the temporal-spatial window in which integration of excitatory postsynaptic potentials (EPSC) can lead to action potential firing.[32] In DMV neurons, this extrasynaptic tonic GABAergic current constitutes the overwhelming majority of the total $GABA_A$ receptor-dependent inhibition,[31] suggesting that volume transmission plays a critical role in determining the excitability of vagal efferent neurons. The origin of GABA activating tonic receptors has been suggested to be completely dependent on action potential-dependent vesicular release in juvenile rats, but does not always require an action potential in mature neurons, suggesting a non-vesicular source of GABA that has not yet been identified.[32]

Previous studies have also shown that GABA transporters (GATs) influence the extent to which GABA can act upon extrasynaptic DMV $GABA_A$ receptors. GATs are Na-Cl symporters that regulate neuronal excitability by modulating extracellular GABA concentration via reuptake of GABA into the pre-synaptic neuron (GAT1) or surrounding glial cells (GAT2/3). These transporters assist in the termination of synaptic transmission, limit the amount of

GABA spillover from synapses, and are responsible for determining extracellular GABA concentration.[33–35] Tonic $GABA_A$ receptors are located peri- and extra-synaptically and their activation reflects extracellular GABA concentration; the activity of GATs, therefore, strongly influences the overall inhibition of DMV neurons.[36] Although GABAergic synapses tend to resist modulation, GABA conductance, hence neuronal excitability, can be altered by changes in neurotransmitter diffusion, location and activity of GATs, activation and location of astrocytes/glia, expression of extrasynaptic receptors, cell maturity amongst other factors.[33–35,37]

Modulation of GABAergic synaptic transmission

Previous studies from this, and other, laboratories, have demonstrated that, under normal conditions, GABAergic synaptic transmission to DMV neurons is relatively resistant to modulation.[38–43] In a series of studies, we demonstrated that the underlying reason for this lack of synaptic modulation is the low level of cAMP-PKA activity within inhibitory synaptic terminals impinging upon DMV neurons. Further, the low ''state of activation'' of these inhibitory terminals is due to tonic activation of presynaptic group II metabotropic glutamate receptors (mGluRs) by glutamate released from vagal afferents which make monosynaptic connections with these inhibitory GABAergic terminals.[44,45] Overcoming this tonic inhibition, either by removal of vagal afferent inputs, antagonism of presynaptic mGluRs, or by activation of adenylate cyclase directly, allows modulation of GABAergic synaptic transmission by a variety of neurotransmitters and neuromodulators including 5-HT, μ-opioid peptides, NPY and PYY, oxytocin (OXT) and insulin amongst others.[38–43] Thus, by increasing activity of the cAMP-PKA second messenger pathway, inhibitory GABAergic synaptic inputs are more susceptible to modulation. By consequence, this results in an increase in availability of presynaptic receptors, which enables the previously unresponsive synapse to be sensitive to neurotransmitters, neuromodulators, or neurohormones. By consequence, the amount of GABA released is altered, hence postsynaptic DMV neuronal excitability is regulated. In contrast, glutamatergic terminals impinging upon DMV neurons appear to have relatively elevated endogenous activity of cAMP-PKA pathways, due to the lack of tonic activation of presynaptic mGluRs; hence, glutamatergic synaptic transmission appears to be always open to modulation.[39,46–48]

Physiological and pathophysiological modulation of GABAergic synaptic transmission

From a physiological perspective, the activity of vagal efferent motoneurons, hence vagal efferent control of gastric motility and tone, is dependent upon prevailing circumstances that may affect cAMP-PKA levels within inhibitory GABAergic vagal neurocircuits. Following a meal, for example, GI neurohormones such as cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1) are released from enteroendocrine cells lining the intestinal mucosa and activate the peripheral terminals of vagal afferents.[49,50] Such GI neurohormones also enter the circulation, and it should be noted that the DVC is known to be a circumventricular organ being surrounded by a leaky blood–brain barrier with permeable fenestrated capillaries.[51,52] Thus, neurons and terminals within the DVC are more vulnerable to peripheral circulating factors and are, indeed, activated by peripheral neurohormones such as CCK.[53] Recent studies have also shown that the ability of exogenous application of insulin to modulate synaptic transmission to DMV neurons in brainstem slice preparations is dependent upon second messenger levels; whether systemic, circulating insulin is capable of the same modulation remains to be elucidated. While, under basal conditions, glutamatergic transmission is decreased by insulin, GABAergic neurons are not initially sensitive to insulin due to low cAMP-PKA levels.[38] After an increase in cAMP-PKA activity, however, insulin decreases GABAergic transmission to gastric-projecting DMV neurons, suggesting that vagal efferent activity, hence gastric functions, will synchronize with feeding/fasting cycles due to the fluctuating levels of hormone and neurohormone/neurotransmitter release. In Type 1 diabetes, however, the absence of insulin may contribute to the lack of appropriate inhibition of this synapse; in addition, recent studies have demonstrated that expression of tonic $GABA_A$ receptors on the membrane surface of DMV neurons of diabetic mice is elevated, further increasing the overall inhibition of the postsynaptic neuron.[38,43,54] Increased GABAergic signaling onto DMV neurons controlling visceral functions would be expected to decrease vagal efferent outflow to several visceral organs including the stomach, pancreas, liver, small intestine, and proximal large intestine. These findings may partly explain the symptoms such as gastroparesis and decreased gastric emptying often seen in Type I and Type II diabetic patients.

Stress is known to exert quite profound effects upon gastric functions via actions at both central and peripheral sites.[55–57] Previous studies have shown that exposure of DMV neurons to corticotropin releasing factor (CRF) is associated with an increase in the cAMP-PKA pathway,[58] modulation of inhibitory GABAergic neurotransmission in DMV neurons, and a decrease in gastric tone and motility (Lewis *et al*., 2002).[98] The hypothalamic anti-stress neuropeptide, OXT, normally inhibits DMV neurons, decreasing in gastric tone and motility; after exposure to CRF, however, OXT inhibits GABAergic transmission, attenuating, or in some cases reversing, the CRF-induced decrease in gastric tone and motility. These studies suggest that CRF modifies the ability of OXT to modulate GABAergic synaptic transmission to DMV neurons, possibly acting to self-regulating the response to central vagal neurocircuits to stress.[5]

Theoretically, the activity of vagal (sensory) afferent inputs will also modulate DMV neuronal activity via activation of presynaptic mGluRs which dampens cAMP levels in inhibitory GABAergic synaptic terminals. Metabotropic glutamate receptors have a much higher affinity for glutamate compared to ionotropic receptors;[59,60] ongoing vagal afferent activity may ensure that sufficient glutamate is released to activate presynaptic mGluRs, even under basal conditions. The excitability and responsiveness of vagal afferent neurons are known to be decreased by diet-induced obesity[8,10,61–63] as

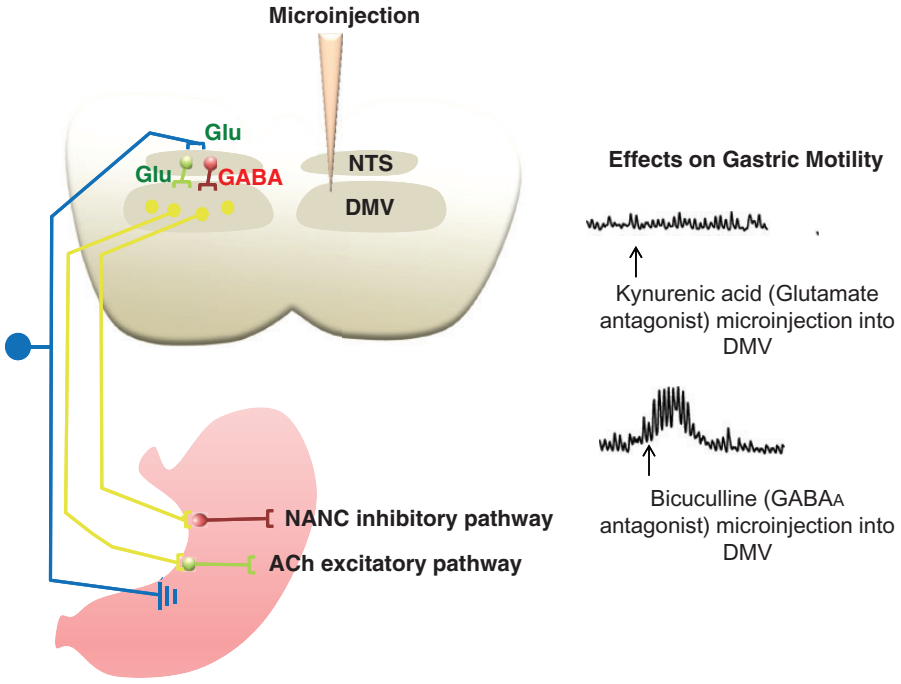

Figure 1 Summary schematic diagram illustrating the prominent role of brainstem GABAergic transmission in regulating vago-vagal reflex control of the stomach. Vagal afferent (sensory; blue) fibers innervating the GI tract transduce and relay signals centrally; the cell bodies of these sensory neurons lie within the paired nodose ganglia and their central terminals enter the brainstem via the tractus solitarius and terminate within the nucleus tractus solitarius (NTS) using predominately glutamate (Glu; green) as their neurotransmitter. NTS neurons integrate these visceral sensory signals with those from other brainstem and higher CNS nuclei involved in autonomic homeostatic control and relay the integrated signal to the adjacent dorsal motor nucleus of the vagus (DMV) using Glu and GABA (red) as neurotransmitters. The critical role of GABAergic signaling to regulate DMV neuronal activity is illustrated by the accompanying gastric motility traces, recorded using miniaturized strain gauges affixed to the ventral surface of the gastric corpus. Note that following microinjection of the non-selective ionotropic glutamate antagonist, kynurenic acid, there was very little change in gastric motility or tone. In contrast, following microinjection of the $GABA_A$ receptor antagonist, bicuculline, gastric motility and tone increased dramatically, demonstrating that DMV neurons are under a tonic inhibitory, GABAergic drive.[27] (A color version of this figure is available in the online journal.)

well as diabetes;[12,64–66] it remains to be determined, however, whether this decrease in activity is sufficient to alter the activation of central mGluRs, and hence inhibitory neurotransmission and the activity of vagal efferent motoneurons. It should be noted, however, that in rats exposed to a high-fat diet from embryonic day 13 onwards, GABAergic transmission to gastric-projecting DMV neurons was able to be modulated even prior to elevation of cAMP levels, suggesting a possible alteration in vagal afferent dependent-activation of mGluRs.[67]

In addition to alterations in gastric functions, disturbances of tonic $GABA_A$ inhibition are also associated with a wide range of psychiatric, neurological and neurodevelopmental conditions including autism spectrum disorder, depression, and cognitive impairments.[68–70] Benzodiazepines, barbiturates, ethanol, and neurosteroids, such as estrogen, all act as allosteric modulators of $GABA_A$ receptor efficacy and/or affinity and therefore exert control over this inhibitory network.[71,72] Low concentrations of ethanol, for example, enhance $GABA_A$ receptor activation, whereas high concentrations of ethanol can activate the receptor independently of GABA.[73] Thus, even in the absence of alterations in $GABA_A$ receptor number or distribution, DMV neurons may respond differently when exposed to these factors, modulating vagal efferent output and with respect to the focus of this review, alter gastric functions.

Developmental modulation of inhibitory neurotransmission in vagal neurocircuits

Is it possible that some of these physiological alterations observed in inhibitory signaling in the brainstem of rats and mice have a developmental origin? Vagal sensory neurocircuits begin development around embryonic day 13 (E13), vagal motoneurons innervating the GI tract can be identified by E14, and central vagal nuclei appear mature by E18.[74–76] Immature neurons have several different characteristics, which are suggested to play an important role in neuronal migration, dendritic arborization, and the formation of synapses.[70,77] Several immunohistochemical and functional studies have demonstrated, however, that vagal neurocircuits continue to undergo a considerable degree of synaptogenesis, synaptic pruning, and reorganization postnatally, and do not appear fully mature until postnatal day 22–28.[74,78,79] Inhibitory brainstem neurosignaling develops during mid-gestation and matures, in rats, around the end of the second postnatal week. During early development, brainstem inhibitory terminals are mixed GABA-glycine synapses.[80] At birth, an increase in GABA axon terminals and the appearance of mixed GABA/glycine axon terminals in the lateral NTS suggests changes in synaptic processing of visceral information in the lateral NTS during postnatal development and is extended into adult neurons;[80] in the adult, however, functional studies have all shown clearly that inhibitory synapses to gastric-projecting DMV neurons

utilize only GABA.[18,21,31,78] Thus, there appears to be a developmental loss of glycinergic transmission in central GI vagal neurocircuits. DMV neurons innervating the GI tract appear to still express functional glycine receptors,[81] suggesting a ''developmental dissociation'' between the loss of glycine as a neurotransmitter but the retention of postsynaptic glycine receptors similar to that reported in other mature central neurons.[82,83] Glycine is well recognized as a principal inhibitory neurotransmitter in the spinal cord and brainstem and is closely involved in cardiorespiratory functions.[84,85] Glycine is often co-released with GABA onto lateral NTS neurons, suggesting a role in visceral information processing.[80] GABA and glycine receptors have similar molecular makeup, consisting of heteropentameric, ligand-gated chloride channels that are members of the cytosine (Cys) loop ion channel receptor family.[86]

In many central neurons, the chloride ion gradient is reversed during early development; high levels of the NKCC1 co-transporter at birth, for example, result in a high intracellular chloride ion concentration, causing GABA and glycine to depolarize neurons. This GABA/glycine-induced depolarization causes a postsynaptic calcium influx that may regulate the gephyrin interactions, which is involved in the clustering of postsynaptic GABA and glycine receptors.[87,88] During maturation, developmental increases in the levels of the KCC2 co-transporter reverse this chloride gradient by the second postnatal week in rats, when GABA induces neuronal hyperpolarization.[37,77,85] Similarly, in many central synapses, both glycine and GABA receptors undergo developmental maturation themselves, which affects channel kinetics; glycine receptors, for example, shift from expressing the $\alpha 2$ subunit at early postnatal time-points to expressing the $\alpha 1$ subunit in mature neurons, shifting the functional properties of the inhibitory synapse to a faster IPSC in the second postnatal week.[89–91] Similarly, the $GABA_A$ receptor in immature neurons contains the kinetically slower $\alpha 2/3$ subunits, while mature neurons contain the faster $\alpha 1/6$ subunits, shifting the functional properties of the inhibitory synapse to a faster inhibitory postsynaptic current in mature neurons.[92] Even in mature neurons, however, glycine and GABA receptor activation have different kinetics, with glycine receptor activation inducing an inhibitory postsynaptic current with a faster IPSC rise time and decay time; thus, the additional use of glycine as a neurotransmitter may allow further integration and fine-tune synaptic inhibition in the postsynaptic neuron.[78,93] Glycine and GABA neurotransmitters act as co-agonists on glycine receptors in the trapezoid body of the brainstem, with glycine acting as a strong agonist and GABA as a weak agonist.[94] Frequently, glycine compliments GABA release and, together, these inhibitory transmitters may influence the generation of early patterns of activity as well as the structure of neural circuits. The integration of glycine and GABA receptor activations allows for adjustments in the time course of inhibitory synaptic transmission, and determines the strength of postsynaptic inhibition, thus optimizing neuronal integration, excitability, and function.

Future directions

The physiological consequences of developmental disruptions in inhibitory brainstem neurocircuit development have yet to be elucidated, but both GABA and glycine appear promising candidates for future research. The perinatal time period is critical for development of vagal neurocircuits and also represents a time when these neurons are vulnerable to different factors, possibly imprinting permanent effects on these neurocircuits.[95–97] Neonatal stress, such as maternal separation for example, has been suggested to influence the development of the GABA transmission in the NTS by altering both pre-synaptic GABA content and postsynaptic $GABA_A$ receptors.[78] It remains to be determined whether and how environmental factors can affect the development of inhibitory synapses which may alter inhibitory signaling in adulthood.[37]

Since the inhibitory network between the NTS and DMV is critical in setting the ''tone'' of vagal efferent outflow, relatively minor changes in development, neurotransmitter release, receptor trafficking, transporter reuptake functions or glial/astrocyte activity, may have dramatic effects on GI outcomes. The question then becomes, which other aspects of the NTS-DMV synapse that have not yet been analyzed can lead to GI dysfunction? Certainly, vagal neurocircuits are open to a wide degree of plasticity, even in adulthood; under what, if any, conditions are these changes in GABAergic signaling permanent? Are these effects reversible? Under what, if any, pathophysiological conditions are GAT functions altered, which would alter extracellular GABA concentration, hence synaptic efficacy? Is it possible that glycine can still be actively co-released in adulthood if the developmental decline in glycinergic transmission is arrested?

While there are still many unanswered questions about the regulation of gastric functions by inhibitory currents onto DMV neurons, identification of the current gaps in knowledge is the first step towards better treatment of the myriad of GI disorders involved in altered vagal signaling. From a clinical perspective, the complex structure and differential subunit composition of GABA and glycine receptors raise the possibility of selective pharmacological and therapeutic interventions in the future.[86,72] Understanding the complexity of inhibitory synaptic regulation of DMV neuronal activity will bring us closer to uncovering the physiology and pathophysiology of autonomic homeostatic control of GI functions.

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