Plasticity of vagal brainstem circuits in the control of gastrointestinal function

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Abstract

The afferent vagus transmits sensory information from the gastrointestinal (GI) tract and other viscera to the brainstem via a glutamatergic synapse at the level of the nucleus of the solitary tract (NTS). Second order NTS neurons integrate this sensory information with inputs from other CNS regions that regulate autonomic functions and homeostasis. Glutamatergic and GABAergic neurons are responsible for conveying the integrated response to other nuclei, including the adjacent dorsal motor nucleus of the vagus (DMV). The preganglionic neurons in the DMV are the source of the parasympathetic motor response back to the GI tract. The glutamatergic synapse between the NTS and DMV is unlikely to be tonically active in regulating gastric motility and tone although almost all neurotransmitters tested so far modulate transmission at this synapse. In contrast, the tonic inhibitory GABAergic input from the NTS to the DMV appears to be critical in setting the tone of gastric motility and, under basal conditions, is unaffected by many neurotransmitters or neurohormones.

This review is based, in part, on a presentation by Dr Browning at the 2009 ISAN meeting in Sydney, Australia and discusses how neurohormones and macronutrients modulate glutamatergic transmission to NTS neurons and GABAergic transmission to DMV neurons in relation to sensory information that is received from the GI tract. These neurohormones and macronutrients appear to exert efficient "on-demand" control of the motor output from the DMV in response to ever-changing demands required to maintain homeostasis.

Keywords

vagus; brainstem; gastrointestinal; plasticity; receptor trafficking

Brainstem autonomic circuitry is critical for integrating homeostatic functions including, amongst other functions, the co-ordination of vago-vagal reflexes and regulation of food ingestion and the functions of the gastrointestinal (GI). Recent data from a number of laboratories, including our own, indicate that vagally-mediated GI functions are dramatically influenced by many GI hormones acting at sites within this brainstem circuitry.

Control of the gastrointestinal tract by vago-vagal reflexes

The central terminals of vagal afferent neurons transmit visceral sensory information to the brainstem through a glutamatergic synapse at the level of the NTS (Andresen & Kunze, 1994;Andresen & Yang, 1990;Hornby, 2001;Travagli *et al.*, 2006;Jean, 2001). Second order NTS neurons display heterogeneous properties and contribute to the integration of synaptic input from vagal afferent axons as well as input from higher centers in the CNS that are involved in regulating autonomic functions and maintaining homeostasis (Bailey *et al.*, 2006;Bailey *et al.*, 2008;Bonham & Chen, 2005;Jin *et al.*, 2004b;Travagli *et al.*, 2006). These converging inputs provide information that is assimilated and integrated with metabolic and hormonal signals to shape the output from the NTS. Among other central autonomic areas, NTS neurons project to the adjacent DMV, where the parasympathetic preganglionic neurons that supply the vagal motor output to the GI tract are located. The cholinergic DMV neurons that control gastrointestinal function innervate postganglionic neurons located within the gastrointestinal tract. Parasympathetic postganglionic neurons are involved in two distinct pathways that control gastric functions. One is an excitatory cholinergic pathway that increases gastric tone, motility and secretion via activation of muscarinic cholinergic receptors. The other is an inhibitory non-adrenergic, noncholinergic (NANC) pathway that inhibits gastric functions mainly by releasing nitric oxide (NO) or vasoactive intestinal polypeptide (VIP) (reviewed in (Travagli *et al.*, 2006). Either activation of the NANC pathway or inhibition of the tonic cholinergic pathway can inhibit gastric function (Figure 1).

Vagal reflexes are critical for the control and integration of visceral functions. As a result, GI pathologies and digestive disorders, including gastroparesis, dyspepsia, esophageal reflux, colitis, anorexia and bulimia nervosa, are often a consequence of, or are associated with, dysfunctional vagal reflexes (Yamano *et al.*, 1997;Saito *et al.*, 2006;Thumshirn, 2002;Ghia *et al.*, 2007;Andrews & Sanger, 2002;Hornby & Abrahams, 2000;Faris *et al.*, 2008).

DMV neurons that project to the stomach are remarkable in that they exhibit slow (1–2 Hz) spontaneous pacemaker-like activity (*in* vitro as well as *in vivo*; (Fogel *et al.*, 1996;Zhang *et al.*, 1992;Travagli *et al.*, 1991) the rate of which can be modulated by synaptic inputs. Although NTS neurons have diverse biophysical and neurochemical properties, functional studies have determined that they provide primarily glutamatergic, noradrenergic and/or GABAergic input to gastric-projecting DMV neurons (reviewed in (Travagli *et al.*, 2006). Gastric tone and motility are increased dramatically by microinjections of GABAergic antagonists into the dorsal vagal complex (DVC = NTS, DMV plus area postrema). In contrast, microinjections of antagonists against glutamate receptors or adrenoceptors have little effect (Sivarao *et al.*, 1998;Travagli *et al.*, 2006;Rogers *et al.*, 2003). These data suggest the existence of a tonic GABAergic input from the NTS to DMV that regulates the firing rate of gastric-projecting DMV neurons and, therefore, gastric vagal motor output.

The observations above also imply that, even in periods of gastric inactivity, vagal efferent outflow to the stomach is continuously sculpted by afferent inputs, including sensory vagal, descending CNS and humoral inputs that modulate the activity of DMV neurons. Vagally-regulated GI functions must exhibit extraordinary adaptive plasticity to ensure an appropriate response to a variety of intrinsic and extrinsic factors, including food, stress and even time of day. An appropriate response is particularly important because even minor changes in neuronal input can influence the inherent pacemaker activity of DMV neurons. Changes in pacemaker activity would alter vagal motor output, causing major changes in gastric motility and tone as well as in responses to vago-vagal reflexes.

Although GABAergic transmission plays a prominent role in brainstem vagal circuits, several groups have been unable to demonstrate modulation of GABAergic synaptic transmition to DMV neurons even by neurotransmitters and neuromodulators known to affect vagal control of gastric functions. This resistance to modulation at such an important synapse led us to question its physiological relevance and benefit. We have begun to address this issue using the well-characterized μ-opioid receptor-effector system, which has a well-defined pharmacology and readily available receptor-selective agonists and antagonists (Browning *et al.*, 2004;Browning *et al.*, 2006). there is also evidence of the same type of effects for serotonin acting at the 5HT-1A receptor (Browning & Travagli, 2001), for oxytocin acting at the OT-1 receptor (Browning *et al.*, 2009), for pancreatic polypeptides acting at Y1 and Y2 receptors (Browning & Travagli, 2009) and for norepinephrine acting at adrenoceptors (Browning and Travagli, unpublished data).

Plasticity at the NTS-DMV synapse depends on cAMP

We have shown that, under basal conditions, low resting levels of cAMP within NTS inhibitory nerve terminals mean that μ-opioid peptides do not modulate GABAergic synaptic transmission to DMV neurons regulating GI function (Browning & Travagli, 2001;Browning *et al.*, 2004;Browning *et al.*, 2002). We subsequently tested the effects of various compounds that raised cAMP levels (forskolin, the satiety peptides CCK or GLP-1, the stress-related neurotransmitters, TRH or CRF, to activate adenylate cyclase directly; IBMX to inhibit phosphodiesterase activity; 8-bromo cAMP, to provide a non-hydrolysable store of cAMP) and "uncovered" the ability of μ-opioid peptides to inhibit transmission at the GABAergic NTS-DMV synapse. In contrast, when we applied drugs that lowered cAMP levels (adenylate cyclase inhibitors, such as dideoxyadenosine) or inhibited the cAMP-PKA pathway (Rp-cAMPs or H89), the ability of adenylate cyclase activators to unmask the modulation of transmission at GABAergic synapses by μ-opioid peptides was abolished.

The effects of manipulating cAMP levels occurred within 5 minutes and disappeared relatively quickly, with the presynaptic inhibition mediated through μ-opioid and polypeptide Y1 & Y2 receptors lasting only about 60 minutes. The rapidity and transience of these effects suggest that *de novo* synthesis of new receptors was not involved in the activation of the cAMP-PKA pathway within GABAergic nerve terminals in the NTS. Instead, the likely mechanism for activation is the trafficking of pre-formed, internalized receptors to the nerve terminal membrane. This conclusion was supported by the effects on cAMP-PKA activation of the Golgi-disrupting agent, Brefeldin-A, or of low temperatures (Browning *et al.*, 2004). Activation of the cAMP-PKA pathway also led to an increase in the occurrence of Gi/o coupled receptors such as μ-opioid receptors (Browning *et al.*, 2004) and OT1 receptors (Figure 2) in terminals that were immunoreactive for GAD_{67}, the GABA synthesizing enzyme, including in GABAergic terminals that closely apposed DMV neurons.

These observations led us to hypothesize that the ability of neurotransmitters or neuromodulators to affect inhibitory transmission to DMV neurons that regulate GI functions is dictated by the level of cAMP (or the level of activity in the cAMP-PKA pathway) in the presynaptic GABAergic nerve terminals. Under basal or resting conditions, when cAMP levels are low, receptors negatively coupled to adenylate cyclase are concentrated inside the synaptic terminal such that neurotransmitters or modulators cannot bind to them and affect GABA transmission. When the cAMP level increases, or the cAMP-PKA pathway is activated in the nerve terminal, the internalized receptors move rapidly and transiently to the membrane of the terminal, permitting other transmitters to bind to their receptors and modulate GABA release.

If this hypothesis is correct, it raises a number of other questions, including: what mechanisms keep levels of cAMP in GABAergic NTS nerve terminals low, and why are GABAergic terminals regulated by cAMP levels but not glutamatergic terminals? To answer these questions we investigated whether the afferent (sensory) vagus plays a role in regulating cAMP levels within GABAergic NTS nerve terminals, since input from higher centers in the CNS is not required for vagally-mediated GI reflexes.

Vagal afferents control cAMP levels in GABAergic terminals in the NTS

We subsequently showed that GABAergic transmission to DMV neurons involved in regulating GI function could be inhibited without increasing cAMP levels (Browning *et al.*, 2006) if we selectively removed vagal afferent inputs either by perivagal application of capsaicin (Holzer *et al.*, 1991) or by surgical rhizotomy. Furthermore, brainstem levels of cAMP were increased by vagal deafferentation as was the co-localization of μ-opioid receptors and markers of GABAergic transmission in nerve terminals in the NTS (Browning *et al.*, 2006) (see also Figure 3, demonstrating the co-localization of OT-1 receptors on GABAergic nerve terminals within the NTS).

These data suggest that vagal afferent inputs do indeed regulate cAMP levels within GABAergic nerve terminals in the NTS and therefore regulate the ability of transmitters to modulate inhibitory synaptic transmission to GI-related DMV neurons. cAMP within nerve terminals are not only important *modulators* of neurotransmitter release, but under certain circumstances, may actually *dominate* the release of neurotransmitter. cAMP can facilitate synaptic transmission by acting at multiple sites, from regulating the availability of intracellular calcium, to the sequence of events leading to exocytosis (Chavis *et al.*, 1998;Ingram *et al.*, 1998;Chavez-Noriega & Stevens, 1994;Leenders & Sheng, 2005). The stimulus-dependent insertion of proteins and receptors into the plasma membrane occurs commonly in many systems, including the CNS (Brismar *et al.*, 1998); (Passafaro *et al.*, 1996;Quick *et al.*, 1997;Shuster *et al.*, 1999). In fact, previously silent synapses have been shown to release neurotransmitters spontaneously following activation of the cAMP/PKA pathway (Chavis *et al.*, 1998). Increasing the intracellular levels of cAMP is also likely to increase the sensitivity of the synapse to activation of receptors negatively coupled to adenylate cyclase (such as μ-opioid, 5-HT1A, pancreatic polypeptide Y1 and Y2 as well as oxytocin OT1 receptors).

As mentioned above, vagal afferent axons use glutamatergic synapses to convey sensory input to the brainstem (Andresen & Kunze, 1994;Andresen & Yang, 1990;Hornby, 2001;Travagli *et al.*, 2006;Jean, 2001). Transmission of this visceral sensory information relies upon the activation of ionotropic NMDA and non-NMDA receptors. Vago-vagal neurocircuits also express metabotropic glutamate receptors (mGluR), indeed mRNA for all 8 mGluR identified to date (mGluR1–8) have been identified in vagal afferent neurons (Page *et al.*, 2005;Hoang & Hay, 2001) and their peripheral terminals and may play prominent roles in gastric and gastroesophageal vagal afferent sensitivity (Page *et al.*, 2005;Young *et al.*, 2008;Young *et al.*, 2007a). Activation of group II mGluR (mGluR2 & 3) and group III mGluR (mGluR4,7,&8), for example, decreases vagal afferent sensitivity and relaxation of the lower esophageal sphincter (Young *et al.*, 2008;Page *et al.*, 2005). In contrast, activation of group I mGluR (mGluR1&5) increases vagal afferent activity and lower esophageal sphincter relaxation; antagonists of mGluR5 in particular may be of benefit in the treatment of esophageal reflux conditions since they appear to exert few, if any, central side effects (Young *et al.*, 2007b).

Centrally, group II and III mGluR are located mainly at presynaptic sites in the DVC and are negatively coupled to adenylate cyclase. Because metabotropic receptors operate through

signaling mechanisms that involve G-proteins, the effects of activation of these receptors on sensory integration and synaptic transmission may be longer-lasting (Hay & Lindsley, 1995;Hay *et al.*, 1999;Glaum & Miller, 1992;Glaum & Miller, 1993;Jin *et al.*, 2004a;Andresen & Yang, 1990;Chen *et al.*, 2002;Chen & Bonham, 2005). Thus, mGluR on GABAergic terminals in the NTS could be critical for regulating cAMP levels and, therefore, the modulation of synaptic transmission. We have demonstrated that the level of cAMP within GABAergic terminals in the NTS depends upon ongoing activation of group II, but not group III, mGluR (Browning & Travagli, 2007). If we applied group II mGluR antagonists to brainstem slice preparations, cAMP levels were elevated sufficiently for μ-opioid receptors to translocate to the terminal membrane such that their activation could inhibit synaptic transmission to GI-related DMV neurons. When we removed glutamatergic inputs by disrupting vagal afferents, these group II mGluR no longer experienced tonic activation and, by consequence, it was no longer necessary to exogenously increase cAMP levels in order to modulate inhibitory synaptic transmission. Although modulating transmission in this way may appear rather unwieldy, this mechanism could be convenient and metabolically "cheap", providing visceral afferent inputs with 'on-demand' control the efferent motor response (Browning & Travagli, 2006;Browning & Travagli, 2007). The principal neurotransmitter that vagal afferent inputs use to control NTS neurons is glutamate. By activating metabotropic, rather than ionotropic, receptors with the same neurotransmitter, vagal afferent inputs can regulate the tonic GABAergic input onto DMV neurons, thereby setting the "tone" of vagal efferent motor output. Using this mechanism, visceral inputs themselves can easily "gate" the efferent output in response to homeostatic demands.

In contrast to GABAergic terminals in the NTS, glutamatergic terminals display both functional group II and group III mGluR (Browning & Travagli, 2007). Thus, the main difference between glutamatergic and GABAergic terminals in the NTS is probably in the monosynaptic input that they receive from vagal afferent axons. There is no tonic activation of either group II or group III mGluR on glutamatergic NTS terminals because they do not appear to receive a direct monosynaptic input from vagal afferents. As a result, there is no suppression of cAMP levels within glutamatergic NTS nerve terminals and their excitatory synaptic transmission to GI-related DMV neurons can be modulated, even in the basal state. Hence, there is likely to be a fundamental difference in vagal afferent-driven glutamatergic transmission to DMV neurons and vagal afferent-driven GABAergic transmission to DMV neurons, with the anatomical organization of vagal brainstem neurocircuitry underlying the two types of transmission being quite distinct (see Figure 4).

Plasticity of vagal brainstem circuits and functional dyspepsia

Functional dyspepsia (FD), or the presence of gastroduodenal symptoms in the absence of any organic, systemic or metabolic explanation, includes a constellation of gastrointestinal symptoms such as impaired gastric emptying, reduced gastric compliance, early satiety and weight loss (Tack *et al.*, 2006). Although the pathophysiology of FD is incompletely understood, there are several indications that vago-vagal reflex control of the upper GI tract is impaired. For example, it has been suggested that FD may from either inadequate vagal sensory stimulation (Lunding *et al.*, 2008;Holtmann *et al.*, 1998;Greydanus *et al.*, 1991) or from excessive or hypersensitive vagal sensory stimulation as a consequence of either insult or injury (Holzer, 2006), including abnormal acid challenge (Holzer, 2003;Holzer *et al.*, 2003).

Although there is variability among subgroups of patients, it has long been known that food ingestion and/or psychosocial factors, such as stress, can aggravate the symptoms of dyspepsia. Interestingly, neurotransmitters, neuromodulators and neurohumoral agents that

are positively coupled to adenylate cyclase, including GLP-1, CCK and CRF, are released in response to both food ingestion and stress; and these neuroactive chemicals ultimately cause an increase in brainstem cAMP levels (Berthoud *et al.*, 2006;Drucker, 2006;Dufresne *et al.*, 2006). Stress-related changes in gastric motility have been identified as one of the main pathophysiological mechanisms underlying dyspeptic symptoms and stressful life events initiate or exacerbate these symptoms in most dyspepsia patients (Tack & Lee, 2005;Mimidis & Tack, 2008). In light of these findings, we have developed the following working hypothesis based on our data and information from other laboratories: vagal afferent terminals release glutamate on NTS neurons that regulate GI function. At "rest" or during periods of minimal feed-back from the GI tract, not enough glutamate is released from vagal terminals to evoke "full-scale" vago-vagal reflexes. However, enough glutamate is released to activate mGluRs present at the NTS-DMV synapse since they have an affinity for glutamate that is several orders of magnitude greater than the affinity of ionotropic receptors. mGluR activation tonically inhibits vagal circuits by decreasing cAMP levels. As a result, the metabolically "inexpensive" neurotransmitter, glutamate, causes a profound and long-lasting dampening of the activity in vago-vagal circuitry.

After ingestion of food or a stressful event, an increase in adenylate cyclase activity induced by circulating hormones or local neurotransmitters activates brainstem vagal circuits. The tonic inhibition induced by mGluR is overcome by the increase in cAMP levels and/or activation of the cAMP-PKA pathway. As a result, $G_{i/o}$ coupled-receptors move to the neuronal membrane of GABA-releasing terminals in the NTS. These trafficked receptors, together with the increase in cAMP, allow neurotransmitters/modulators to modulate the tonic GABAergic input from the NTS to DMV neurons. As a result, the vagal efferent outflow to the viscera can now undergo the adaptive changes that are necessary to prepare the GI tract for the appropriate ingestive and digestive responses. In functional dyspepsia patients, however, these adaptive changes do not occur and disrupted coupling in the sensory-motor vagal circuitry leads to altered gastric motility. In fact, these circuits may be in a constant "state of activation" in functional dyspepsia due to the enhanced release (or coupling) of stress- or meal-related hormones. These hormones increased cAMP levels in vago-vagal circuits, overcoming the dampening of effects mGluR on the GABAergic synapses. By consequence, signals from an otherwise harmless situation, such as meal ingestion, that usually induce plasticity and adaptability are processed inappropriately and gastric functions are disrupted.

Plasticity at the vagal afferent-NTS synapse depends on glucose

Plasticity within GI vagal brainstem circuits may not be restricted to the GABAergic synapse between the NTS and DMV. Recent evidence suggests that the first synapse in vago-vagal reflexes, i.e., the synapse between vagal afferent terminals and NTS neurons may be open to modulation (Wan & Browning, 2008a;Wan & Browning, 2008b). In fact, glutamatergic transmission from vagal afferent terminals increases or decreases with an elevation or reduction in extracellular glucose levels, respectively (Wan & Browning, 2008a). This effect of glucose to regulate excitatory synaptic transmission appears to involve alterations in either the number and/or the function of presynaptic 5-HT3 receptors, which act to facilitate glutamate release (Wan & Browning, 2008b) (Figure 5).

While they are undoubtedly lower than peripheral glucose levels, central glucose levels do appear to change in concert with blood glucose levels although over a much narrower range (Silver & Erecinska, 1994;Dunn-Meynell *et al.*, 2009). Furthermore, the area postrema is entirely outside the blood-brain barrier and the NTS and DMV have a 'leaky' blood-brain barrier because of their fenestrated capillaries (Cottrell & Ferguson, 2004;Gross *et al.*, 1990). Circulating factors can therefore more easily access neurons within the DVC and

glucose levels within the NTS or DMV may actually be higher than in other central nuclei (Routh, 2002). Our preliminary evidence supports the idea that more "physiological" levels of extracellular glucose modulate glutamatergic transmission from vagal afferent terminals (Figure 5).

Plasticity of vagal brainstem circuits and gastric emptying

Vagal afferents regulating the GI tract are likely to be excited by glucose acting at a variety of central and peripheral sites. These would include peripheral vagal terminals within the intestine (Raybould & Zittel, 1995;Raybould *et al.*, 2003), nerve cell bodies in the nodose ganglion (Grabauskas *et al.*, 2006) and central vagal terminals within the brainstem (Wan & Browning, 2008a). Rather than redundancy, these multiple sites of action may provide a large safety margin for signal processing. Alternatively, glucose acting at multiple sites could amplify peripheral signals to produce a rapid and robust response in motor output. Gastric relaxation and a delay in gastric emptying result from glucose within the intestinal tract (Gentilcore *et al.*, 2007;Rayner *et al.*, 2001) so vagal afferent activation by glucose is likely to cause an inhibitory efferent motor response.

The precise mechanisms that underlie glucose-induced gastric inhibition remain controversial. As discussed above, an increase in the activity of the postganglionic parasympathetic NANC efferent pathway or an inhibition of the cholinergic efferent pathway could cause vagally-mediated inhibition of gastric function (Travagli *et al.*, 2006) and published studies support the involvement of both pathways (Travagli *et al.*, 2006) Part of the controversy stems from the different experimental methods used in these studies, but there are also problems with distinguishing the influence of peripheral versus central glucose effects on GI functions. In fact, it is possible that both cholinergic efferent and NANC efferent pathways are involved in glucose-dependent gastroinhibition. Glucose in the intestine, and its absorption into the blood, could first initiate vago-vagal reflexes through activation of NANC pathways. Subsequently, when glucose levels in the brainstem have risen, inhibition of cholinergic efferent pathways could cause a temporally distinct, prolonged direct gastroinhibition (Figure 6).

Conclusions & Future Directions

Several laboratories have recently provided conclusive evidence that vagally-mediated GI reflexes do not rely on static neuronal circuits that produce stereotypical output responses to visceral signaling. Instead, these reflex pathways exhibit a high degree of modulation and plasticity. Within the brainstem circuits that regulate GI function, parasympathetic motor output can be modulated by visceral information, which can consists of either vagal afferent activity itself, GI-related neurohormones released in response to meal ingestion, macronutrients or a combination of these factors. GI responses are timed over either minutes or hours so that the response is appropriate to the homeostatic demands placed upon the gastrointestinal tract. These features of brainstem GI circuitry are clearly different to the circuitry mediating other autonomic reflexes. Hypertension, for example, causes modulation and plasticity in brainstem baroreflex circuits over much longer periods of time (1–5 weeks) (Tang & Dworkin, 2007b;Tang & Dworkin, 2007a;Hermes *et al.*, 2008;Mei *et al.*, 2003;Zhang *et al.*, 2008). These differences support the long-standing idea that vagal pathways in the brainstem are organized into circuits that are anatomically and functionally specific.

Clearly, there is still much to be learned about the plasticity of vagal brainstem circuits. Serious long-term health consequences can result from malfunctioning GI vago-vagal reflexes but the precise mechanism(s) responsible for breakdowns in autonomic and

homeostatic regulation remain to be discovered. Tonic vagal afferent input is obviously critical for setting output "tone" but the effects of long-term increases or decreases in vagal activity have not yet been investigated. Similarly, we know little about the effects of long-term alterations in the release of GI neurohormones, such as CCK and GLP-1, on vagally-mediated reflexes. The plasticity in vagal circuitry discussed here is readily reversible. Could more permanent re-modeling of brainstem vagal circuitry underlie chronic GI disturbances, such as functional dyspepsia or persistent reflux; and what circumstances would evoke more persistent remodeling? Is the "gain" of plasticity set during development and what processes might disrupt this gain-setting? Are all second messenger systems within vago-vagal brainstem circuits subject to plasticity? Clearly, these questions and many others must be answered before we can understand the role of vagal reflexes and plasticity in autonomic control of homeostasis.

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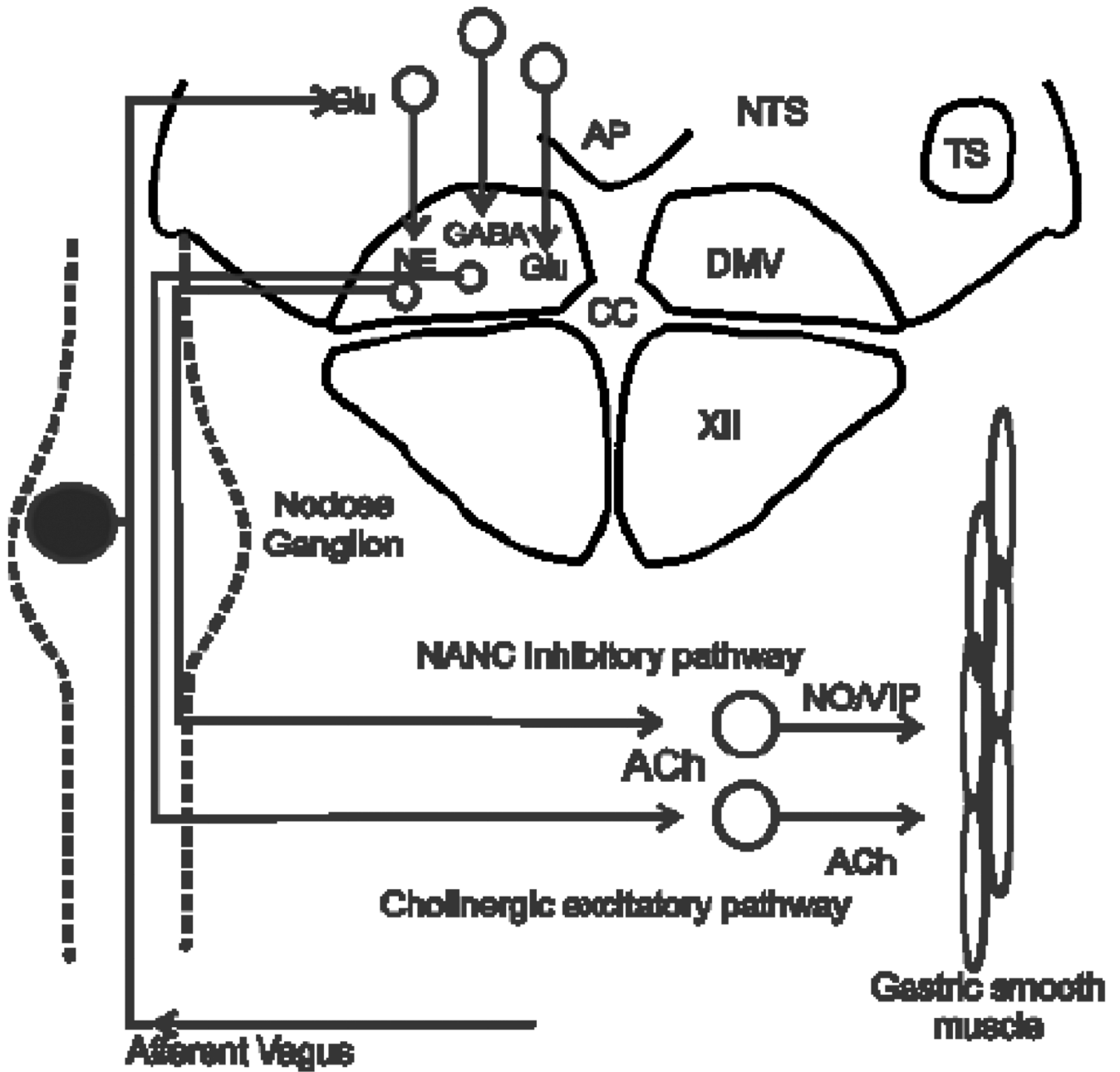
Figure 1. Schematic diagram illustrating vago-vagal reflex control of the stomach
The afferent vagus nerve relays sensory information from the GI tract centrally through cell
bodies of sensory neurons in the paired nodose ganglia. Afferent axons carrying GI-related
signals enter the dorsal medulla through the solitary tract (TS) and terminate within the
NTS, where they use glutamate as their main neurotransmitter. Visceral afferent signals are
integrated with inputs from other brainstem and higher CNS nuclei by NTS neurons. The
resultant response is transmitted to the adjacent DMV, which contains the preganglionic
parasympathetic motor neurons that provide the principal motor output to the stomach, and
other central regions.
DMV neurons are cholinergic, and activate nicotinic cholinergic receptors on postganglionic
neurons within the stomach. Two distinct postganglionic pathways are involved in

regulating gastric activity. A cholinergic excitatory pathway increases motility and tone through activation of muscarinic cholinergic receptors on gastric smooth muscle. A non-adrenergic, non-cholinergic pathway decreases gastric tone and motility by releasing mainly nitric oxide (NO) and vasoactive intestinal polypeptide (VIP). Thus, either withdrawal of tonic cholinergic activity or via activation of the non-adrenergic, non-cholinergic (NANC) pathway can inhibit gastric functions.
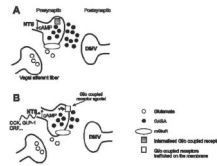
Figure 2. Schematic diagram illustrating the cAMP-dependent trafficking of Gi/o coupled receptors on GABAergic NTS terminals

GABAergic NTS neurons provide a tonic inhibitory input onto vagal efferent DMV motoneurons. Glutamate released from vagal afferent (monosynaptic) terminals activates mGluR present on GABAergic terminals, decreasing cAMP levels within the nerve terminal (A). This, in turn, results in internalization of Gi/o coupled receptors, such as μ-opioid, oxytocin (OT-1), 5-hydroxytryptamine 5-HT$_{1A}$, neuropeptide Y1 and Y2 receptors, removing their ability to modulate inhibitory synaptic transmission between NTS and DMV. Exposure to neurohormones such as the satiety peptides cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) or the stress neurotransmitter corticotrophin releasing factor (CRF) activates adenylate cyclase and increases cAMP levels within the nerve terminal, overcoming the effects of metabotropic glutamate receptor (mGluR) activation (B). This results in trafficking of Gi/o coupled receptors to the nerve terminal, allowing their activation to modulate inhibitory synaptic transmission to DMV neurons.
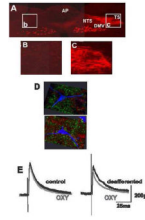
Figure 3. Deafferentation increases co-localization of oxytocin receptors on GABAergic nerve terminals within the brainstem and allows modulation of inhibitory synaptic transmission between the NTS and DMV

A, B,C: Anatomical confirmation of successful deafferentation surgery (rootlet rhizotomy) in a rat that received an injection of rhodamine dextran into the corpus. Coronal section through the rostral dorsal vagal complex (DVC). Note that rhodamine dextran-labeled vagal afferent terminals are absent from the solitary tract (TS) or NTS (A,B) on the deafferented side of the brainstem (left). In contrast, a dense vagal afferent innervation is present the intact side of the brainstem (right) receives (A,C). Also note that both right and left DMV contain labelled preganglionic motoneurons, confirming that the surgery is selective for vagal afferents.

D: Brainstem sections containing the DMV were immunohistochemically processed to reveal the oxytocin-1 receptor with a red fluorophore and GAD67 (γ-aminodecarboxylase; the enzyme required for GABA synthesis, hence used as a marker for GABAergic nerve terminals), with a green fluorophore. Note that, in the deafferented brainstem (upper micrograph) oxytocin-1 receptors (red) and GAD (green) are often co-localized (yellow; arrows) and that some of the GAD terminals containing oxytocin receptors appose a Neurobiotin-filled DMV neuron (blue). In contrast, in a vagally-intact brainstem (lower micrograph), few, if any, terminals contain co-localized GAD and oxytocin-1 receptors.

E: Whole cell patch clamp recordings of DMV neurons voltage clamped at −50mV from control (left) and deafferented (right) brainstems. electrical stimulation of the adjacent NTS evoked GABAergic inhibitory currents in the recorded neurons.. In vagally-intact brainstems, oxytocin (100nM) did not affect the amplitude of evoked GABAergic currents (left) whereas, in deafferented brainstems, the amplitude of evoked GABAergic currents in DMV neurons was decreased by the application of oxytocin.

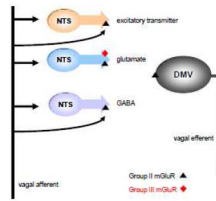Adapted, in part, from Browning et al. 2004 & (Wan *et al.*, 2008).

Figure 4. Proposed schematic representation of the location of groups II and III mGluR within gastric brainstem circuits
Within the rat brainstem, GABAergic, glutamatergic and other excitatory nerve terminals impinging upon gastric-projecting DMV neurons express presynaptic group II mGluR. These receptors are also present on a subpopulation of DMV neurons. Glutamatergic nerve terminals also express Group III mGluR. GABAergic but not glutamatergic terminals receive monosynaptic glutamatergic input from vagal afferent terminals that is tonically active. Previous studies (Browning & Travagli, 2007) suggest that non-glutamatergic excitatory terminals impinging upon gastric-projecting DMV neurons may also receive a tonically-active monosynaptic input from vagal afferent terminals. Adapted from Browning & Travagli, J. Neuroscience 2007.
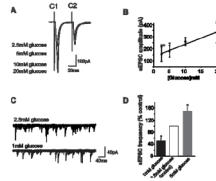
Figure 5. Glucose modulates glutamatergic synaptic transmission

A: Representative traces showing that electrical stimulation of the solitary tract evoked paired glutamatergic excitatory postsynaptic currents (EPSCs), marked C1 and C2, in an NTS neuron voltage clamped at −60 mV. Glucose modulated the amplitude of the evoked EPSC was in a concentrationdependent manner.

B: Graphical representation showing that glucose concentration correlates with EPSC amplitude. *P<0.05 vs 10mM glucose; **P<0.05 vs 20mM glucose.

C: Six consecutive traces from an NTS neuron voltage clamped at −60mV showing that The frequency, but not the amplitude, of spontaneous EPSCs was decreased by lowering the extracellular glucose concentration from 2.5mM to 1.0 mM.

D: Graphical representation showing that glucose concentration correlates with the frequency of spontaneous EPSC. Note that "physiological" changes in the concentration of extracellular glucose also alter glutamatergic synaptic transmission to NTS neurons. *P<0.05 vs control.
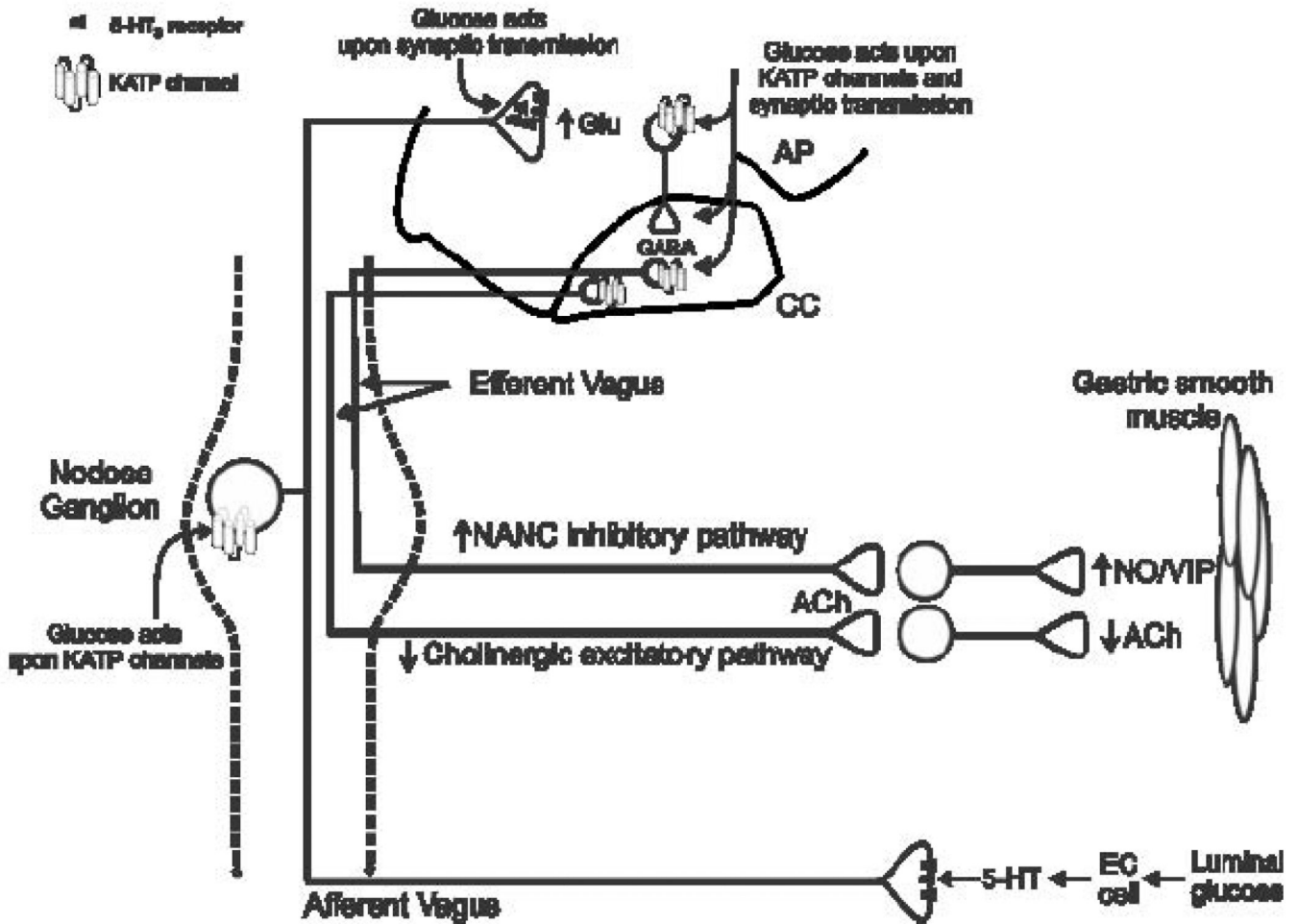
Adapted from Wan & Browning, 2008

Figure 6. Schematic diagram illustrating the actions of glucose on vago-vagal reflexes controlling the stomach
Glucose in the intestinal tract induces the release of serotonin from enterochromaffin (EC) cells(Freeman *et al.*, 2006;Zhu *et al.*, 2001). The released 5-HT acts upon 5-HT$_3$ receptors present on the peripheral terminals of vagal afferent neurons to induce excitation (Savastano *et al.*, 2005;Hillsley *et al.*, 1998;Grundy, 2006); this peripheral signal is presumably relayed centrally via the afferent vagus nerve. Glucose, however, is also able to act directly upon vagal afferent neurons contained within the nodose ganglion via actions upon KATP channels to induce neuronal excitation (Grabauskas *et al.*, 2009). Centrally, glucose acts to increase glutamate release from vagal afferent terminals (Wan & Browning, 2008a) by a mechanism that appears to involve 5-HT$_3$ receptors(Wan & Browning, 2008b)). Glucose can also activate NTS neurons via actions upon KATP channels and increase synaptic transmission to gastric-projecting DMV neurons (Ferreira, Jr. *et al.*, 2001;Shi *et al.*, 2003). Glucose can also act directly upon DMV neurons to alter their excitability (Balfour & Trapp, 2007;Balfour *et al.*, 2006;Trapp *et al.*, 1994). The result of these peripheral and central actions is gastric relaxation and delayed gastric emptying (Raymer *et al.*, 2000;Rayner *et al.*, 2001;Schvarcz *et al.*, 1997) which may involve both withdrawal of cholinergic excitation (Shi *et al.*, 2003;Shi *et al.*, 2005) as well as activation of non-adrenergic, non-cholinergic (NANC) efferent vagal pathways (Zhou *et al.*, 2008).