Role of central vagal 5-HT \textsubscript{3} receptors in gastrointestinal physiology and pathophysiology

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Vagal neurocircuits are vitally important in the co-ordination and modulation of GI reflexes and homeostatic functions. 5-hydroxytryptamine (5-HT; serotonin) is critically important in the regulation of several of these autonomic gastrointestinal (GI) functions including motility, secretion and visceral sensitivity. While several 5-HT receptors are involved in these physiological responses, the ligand-gated 5-HT \textsubscript{3} receptor appears intimately involved in gut-brain signaling, particularly via the afferent (sensory) vagus nerve. 5-HT is released from enterochromaffin cells in response to mechanical or chemical stimulation of the GI tract which leads to activation of 5-HT \textsubscript{3} receptors on the terminals of vagal afferents. 5-HT \textsubscript{3} receptors are also present on the soma of vagal afferent neurons, including GI vagal afferent neurons, where they can be activated by circulating 5-HT. The central terminals of vagal afferents also exhibit 5-HT \textsubscript{3} receptors that function to increase glutamatergic synaptic transmission to second order neurons of the nucleus tractus solitarius within the brainstem. While activation of central brainstem 5-HT \textsubscript{3} receptors modulates visceral functions, it is still unclear whether central vagal neurons, i.e., nucleus of the tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMV) neurons themselves also display functional 5-HT \textsubscript{3} receptors. Thus, activation of 5-HT \textsubscript{3} receptors may modulate the excitability and activity of gastrointestinal vagal afferents at multiple sites and may be involved in several physiological and pathophysiological conditions, including distention- and chemical-evoked vagal reflexes, nausea, and vomiting, as well as visceral hypersensitivity.

Keywords: vagus, vagal afferent, 5-HT, plasticity, gastrointestinal

VAGO-VAGAL REFLEX CONTROL OF GI TRACT

Despite intrinsic (enteric) neural plexuses that allow a considerable degree of autonomy over digestive functions, the central nervous system (CNS) provides extrinsic neural inputs to the GI tract that govern, regulate and modulate these functions. The GI tract receives extrinsic neural inputs from both parasympathetic and sympathetic pathways derived (or controlled) from caudal brainstem nuclei (Browning and Travagli, 2014). While the sympathetic nervous system exerts a predominantly inhibitory effect upon GI muscle and mucosal secretion and regulates GI blood flow via neurally-dependent vasoconstriction, the parasympathetic nervous system exerts both excitatory and inhibitory control over gastric and intestinal motility and tone suggesting a more finely tuned regulation of GI functions (Travagli et al., 2006). The esophagus, stomach, and upper GI tract, in particular, receive a dense parasympathetic innervation, the intensity of which decreases as one progresses distally through the intestine (Berthoud et al., 1991).
The parasympathetic innervation to the stomach, small intestine and proximal colon is provided by the vagus nerve. A mixed nerve, containing both sensory and motor fibers, the vagus contains approximately 70–80% sensory fibers that transduce physiological events within the GI tract and relay this information to the CNS. Anatomical and physiological studies have characterized several different types of vagal afferent fibers that can be distinguished based upon their responses to muscle tension or pressure (mostly low-threshold mechanosensors although high-threshold nociceptors are also present), the location of the afferent fibers receptive field (muscle, mucosal, or serosal/mesenteric) and their principle stimulus modality (chemical, osmotic, mechanical) as well as the region of the GI tract they innervate (Powley and Phillips, 2002; Beyak and Grundy, 2005).

The cell bodies of vagal sensory afferents, which lie within the paired nodose ganglia or nodose-jugular complex, serve the classic afferent functions and relay the peripheral sensory information from the GI tract to the brainstem via a glutamatergic synapse at the level of the nucleus tractus solitarius (NTS). NTS neurons assimilate this enormous volume of sensory information and integrate it with inputs received from other brainstem and higher CNS centers involved in autonomic homeostatic functions. Indeed, the NTS has either reciprocal connections with, or receives inputs from, the hypothalamus, amygdala, nucleus accumbens, raphe, trigeminal, vestibular, and parabrachial nuclei as well as the area postrema, all of which help to sculpt and shape these vagal afferent visceral sensory inputs. The integrated signal is then relayed from the NTS to the adjacent dorsal motor nucleus of the vagus (DMV) which contains the preganglionic parasympathetic motorneurons which supply the parasympathetic output to the upper GI tract via the efferent vagus nerve (Figure 1: Travagli et al., 2006; Browning and Travagli, 2014).

5-HT$_3$ RECEPTORS AND VAGAL SENSORY FUNCTIONS

5-HT is an important neurotransmitter in several GI functions, and >90% of the total body 5-HT is contained with the GI tract, either within specialized enteroendocrine cells, termed enterochromaffin (EC) cells or within neurons. Excellent recent reviews have provided in depth coverage of the role of 5-HT within the GI tract (Gershon and Tack, 2007; Mawe and Hoffman, 2013); this review, therefore, will concentrate on the role of 5-HT$_3$ receptors in gut-brain and brain-gut signaling outside the GI tract itself. Electrophysiological studies have demonstrated functionally active 5-HT$_3$ receptors on vagal afferent neurons and fibers (Leal-Cardoso et al., 1993; Hillsley et al., 1998; Kreis et al., 2002; Moore et al., 2002; Lacolley et al., 2006a; Babic et al., 2012) and activation of 5-HT$_3$ receptors induces a short latency, transient increase in firing rate of vagal afferents (Hillsley and Grundy, 1998; Hillsley et al., 1998) or a brief, rapid inward current (or membrane depolarization), in isolated neurons (Leal-Cardoso et al., 1993; Peters et al., 1993; Babic et al., 2012) consistent with its function as a ligand-gated cation channel (Derkach et al., 1989).

When released from EC cells, 5-HT triggers smooth muscle activity via activation of 5-HT$_3$ receptors on intrinsic primary afferent neurons (IPANs; Tuladhar et al., 1997; Zhou and Galligan, 1999; Bertrand et al., 2000; Gwynne and Bornstein, 2007). Such motor responses can, and do, activate extrinsic vagal and spinal afferent fibers, possibly via 5-HT$_3$ receptors secondary to smooth muscle activity (Blackshaw and Grundy, 1993; Hillsley and Grundy, 1998; Hillsley et al., 1998). The released 5-HT also activates extrinsic primary afferent terminals directly, however, via activation of 5-HT$_3$ receptors (Paintal, 1951; Hillsley et al., 1998). EC cells release 5-HT in response to mechanical (Bulbring and Lin, 1958; Blackshaw and Grundy, 1993; Mazda et al., 2004) as well as chemical stimulation. Luminal micronutrient content, in particular carbohydrates and hyperosmotic stimuli, induce strongly the release of 5-HT (Raybould and Zittel, 1995; Zhu et al., 2001; Raybould et al., 2003; Wu et al., 2005). Vagal afferent nerve terminals innervate the apical tips of mucosal villi as well as intestinal crypts and are likely, therefore, to be in close apposition to GI neurohormones, including 5-HT, released from mucosal enteroendocrine cells (Powley et al., 2011). While a large proportion of the 5-HT from EC cells may be released in close proximity to 5-HT$_3$-containing primary afferent terminals, a significant amount is still absorbed into the bloodstream, and circulating platelet-free 5-HT levels rise almost three-fold following a meal (Houghton et al., 2003). In this regard, it is important to note that (1) 5-HT$_3$ receptors are also present on nodose neuronal membranes (Leal-Cardoso et al., 1993; Moore et al., 1999, 2002; Lacolley et al., 2006a), including those innervating the GI tract (Daly et al., 2011; Babic et al., 2012), (2) 5-HT$_3$ receptors are also present on the central terminals of vagal afferents within the brainstem (Glaum et al., 1992; Ramage and Mifflin, 1998; Wan and Browning, 2008b; Takenaka et al., 2011; Cui et al., 2012; Hosford et al., 2014), and (3) circulating mediators have far freer access to vagal soma and the brainstem than perhaps thought previously (Figure 1; Lacolley et al., 2006b; Baptista et al., 2007). This suggests that EC-derived circulating 5-HT has the potential to modulate vagal afferent neuronal activity at sites distinct from the GI tract and may, therefore, prolong or amplify local GI signaling.

Within the brainstem, activation of 5-HT$_3$ receptors on vagal afferent terminals increases glutamatergic transmission to second order NTS neurons causing their activation (Glaum et al., 1992; Jeggo et al., 2005; Wan and Browning, 2008b; Takenaka et al., 2011; Cui et al., 2012; Hosford et al., 2014). NTS neurons are critically important in the regulation and modulation of a wide variety of autonomic homeostatic functions including cardiovascular as well as gastrointestinal processes (Andresen and Kunze, 1994; Travagli et al., 2006). Activation of vagal 5-HT$_3$ receptors has been shown to be important in in baroreceptor and chemoreceptor reflex control of the cardiovascular system (Sévoz et al., 1996, 1997; Callera et al., 1997; Jeggo et al., 2005; Jordan, 2005; Ramage and Villalon, 2008) as well as pancreatic secretion (Mussa et al., 2008, 2010), meal termination, early satiety, and appetite regulation (Hayes and Covasa, 2006a; Wu et al., 2012).

The source of 5-HT activating 5-HT$_3$ receptors on the central terminals of vagal afferents is the subject of some debate. 5-HT$_3$ receptor selective antagonists decrease glutamatergic synaptic transmission from central vagal afferent terminals (Wan and
FIGURE 1 | Schematic illustration of the location of 5-HT$_3$ receptors on vagal neurocircuits. 5-HT is released from intestinal enteroendocrine cells in response to ingested carbohydrates and acts locally on 5-HT$_3$ receptors present on vagal afferent peripheral terminals to increase vagal afferent fiber firing. Circulating 5-HT may also modulate vagal afferent fiber excitability via actions at 5-HT$_3$ receptors on the soma of subpopulations of nodose ganglion neurons, as well as the central terminals of vagal afferent fibers within the brainstem. Some nodose ganglion neurons are themselves serotonergic, although it is unclear whether they release 5-HT in a physiologically-relevant manner; serotonergic medullary raphe neurons are an additional potential source of 5-HT input into vagal brainstem neurocircuits. An increase in vagal afferent fiber excitability, as results from activation of 5-HT$_3$ receptors, increase glutamatergic transmission to second order NTS neurons. It is unclear whether NTS and DMV neurons themselves display functional 5-HT$_3$ receptors or whether the observed alterations in their activity is subsequent to the modulation of vagal afferent fiber neurotransmission. Browning, 2008b; Cui et al., 2012; Hosford et al., 2014) suggesting the receptors are active tonically, although other studies have not observed this ongoing receptor activation (Cui et al., 2012). Such disparities may be explained by either experimental differences, since tonic 5-HT$_3$ receptor activation was noted in studies employing coronal rather than horizontal brainstem slices, or species differences, being noted in studies involving rats, rather than mice. Immunohistochemical studies have demonstrated a dense serotonergic input into the dorsal vagal complex (i.e., NTS, DMV, and area postrema from the raphe nuclei (Steinbusch, 1981; Thor and Helke, 1987, 1989) the projections of which are more likely to remain intact in the coronal plane. It should also be noted, however, that the dorsal vagal complex is essentially a circumventricular organ with fenestrated capillaries and a leaky blood brain barrier (Cottrell and Ferguson, 2004; Fry and Ferguson, 2007) and circulating neurohormones or neuromodulators may have free access to neurons within these areas (Baptista et al., 2007). It remains to be determined, however, whether elevations in circulating platelet-free 5-HT levels that occur in response to meal ingestion or mechanical stimulation exert any modulatory role on central vagal afferent neurotransmission. It should also be noted, however, that a subpopulation of nodose ganglion neurons have been shown to synthesize 5-HT (Gaudin-Chazal et al., 1982; Thor et al., 1988; Nosjean et al., 1990), although it is unclear whether vagal afferents are able to release 5-HT centrally under physiological conditions.

5-HT$_3$ RECEPTORS AND VAGAL MOTOR FUNCTIONS

Surprisingly, it is not clear whether NTS and DMV neurons themselves display functional 5-HT$_3$ receptors. Extracellular brainstem recordings have certainly demonstrated an alteration in NTS and DMV neuronal activity in response to both peripheral and central administration of 5-HT$_3$ receptor agonists (Wang et al., 1996; Pires et al., 1998; Jeggo et al., 2005; Ramage...
and Villalon, 2008) while nerve recordings have demonstrated that vagal afferent activity is modulated following activation of 5-HT3 receptors (Mussa et al., 2010). The location of these 5-HT3 receptors has not been elucidated precisely; electron microscopy has shown that 5-HT3 receptors are present on neurons and glial cells within the brainstem suggesting an involvement in modulating postsynaptic neuronal responses as well presynaptic neurotransmitter release (Huang et al., 2004). Indeed, one relatively early study (Glaum et al., 1992) demonstrated that NTS neurons were depolarized by exogenous application of a 5-HT3 receptor agonist in a manner resistant to synaptic blockade, suggesting a postsynaptic receptor location. The alteration in neuronal activity in the majority of the remaining studies, however, could conceivably be the downstream response following increased glutamate release subsequent to activation of vagal afferent terminal 5-HT3 receptors.

**PHYSIOLOGICAL ROLES OF VAGAL 5-HT3 RECEPTOR SIGNALING**

The physiological, rather than pathophysiological, role of vagal afferent 5-HT3 receptors following GI-mechanical or distention-related 5-HT release appears to still be open to debate. Several studies have demonstrated that mechanical stimulation of the GI tract activates vagal afferents; some studies describe this as direct activation of peripheral primary afferent 5-HT3 receptors (Mazda et al., 2004; Hayes and Covasa, 2006b), while others show this clearly to be an indirect effect, secondary to stimulation of local motor activity in response to the released 5-HT (Blackshaw and Grundy, 1993; Hillsley and Grundy, 1998; Hillsley et al., 1998). Indeed, recent work has suggested that while release of 5-HT from intestinal EC cells may not be a requirement for either the initiation or propagation of colonic motor complexes, 5-HT certainly modulates these peristaltic reflexes in a manner that appears to involve 5-HT3 receptors (Keating and Spencer, 2010; Spencer et al., 2011).

In contrast, chemically-stimulated 5-HT release has well-defined actions to activate vagal afferent 5-HT3 receptors directly. Ingestion of carbohydrates such as glucose, for example, induces a vagally-dependent gastric relaxation and delay in gastric emptying that is dependent upon peripheral vagal afferent 5-HT3 receptor activation; furthermore, peripheral application of 5-HT3 receptor selective agonists decrease gastric motility and delay gastric emptying (MacGregor et al., 1976; Rayner et al., 2001; Zhu et al., 2001; Raybould et al., 2003). Indeed, peripheral vagal afferent 5-HT3 receptor activation appears to play an ongoing modulatory role in the regulation of gastric motility and emptying since administration of 5-HT3 receptor selective antagonists accelerates gastric transit, suggesting the receptors may be under some degree of tonic activation (Coleman et al., 2003; Raybould et al., 2003; Gentilcore et al., 2007).

The physiological role that 5-HT3 receptors on the central terminals of vagal afferents plays in the glucose-induced, vagally-dependent decrease in gastric motility and tone has still to be elucidated. Studies have demonstrated, however, that the response of vagal afferents to ingested glucose can be modulated by intravenous glucose (Mei, 1978) implying that glucose is capable of modulating vagal activity at sites other than afferent terminals within the GI tract. Indeed, studies have shown that a some GI-vagal afferent neurons are glucose-sensitive, that is, glucose can modulate the excitability of a subpopulation of GI nodose ganglion neurons via actions at ATP-sensitive potassium channels, in a manner similar to the canonical model of pancreatic β-cells (Grabauskas et al., 2010). This implies that, in addition to increasing vagal afferent activity via 5-HT release and subsequent 5-HT3 receptor activation, once absorbed form the GI tract, circulating glucose may also regulate nodose neuron excitability to modify the increase in vagal activity induced by luminal glucose. In addition to these actions of glucose, however, we have demonstrated that extracellular glucose levels are also able to modulate the density and function of 5-HT3 receptors on GI nodose neurons. In particular, increasing extracellular glucose levels induces the trafficking of existing 5-HT3 receptors to the membrane of GI-projecting vagal afferent neurons and increases the magnitude of the 5-HT-induced inward current, whereas decreasing glucose levels induce 5-HT3 receptor internalization and decrease the 5-HT-dependent inward current (Babic et al., 2012). Thus, ingested glucose may be able to amplify and prolong its afferent signaling by first releasing 5-HT from intestinal EC cells, and then by increasing the number of 5-HT3 receptors on vagal afferents available for activation.

The glucose-dependent modulation of 5-HT3 receptor trafficking and function also appears to occur centrally. We, and others, have demonstrated that extracellular glucose regulates the density of 5-HT3 receptors on vagal afferent central terminals; elevating extracellular glucose increases spontaneous and evoked glutamate release from vagal afferent terminals via actions in a 5-HT3 receptor-dependent manner (Wan and Browning, 2008a; Hosford et al., 2014) although the role of vagal afferent 5-HT3 receptors in the glucose-dependent modulation of gastric functions remains to be defined. Similarly, the concentration of glucose within the NTS parenchyma, and fluctuations in response to alterations in circulating glycemic levels, remain to be determined but concentrations within the cerebrospinal fluid are typically two-thirds those of circulating levels. As discussed previously, the dorsal vagal complex is a circumventricular organ and NTS neurons and fiber terminals may well be exposed to higher glucose levels than those measured elsewhere within the CNS (Dunn-Meynell et al., 2009). While the majority of electrophysiological studies in brainstem slice preparations certainly use non-physiological levels of glucose, we have demonstrated previously that glucose modulates glutamate release from vagal afferent terminals at much lower levels of extracellular glucose (0.5–5 mM; Browning, 2013) implying this is a physiological, rather than pathophysiological, phenomenon.

**PATHOPHYSIOLOGICAL ROLES OF VAGAL 5-HT3 RECEPTOR SIGNALING**

The sensory vagus nerve is generally considered to relay predominately non-noxious, interoceptive information from the
GI tract to the brainstem although growing evidence suggests the involvement of the vagus nerve in pain processing (see Randich and Gebhart, 1992). Certainly, some vagal afferent fibers appear responsive to nociceptive stimulation although the primary response to noxious vagal afferent stimulation may be nausea, rather than pain (Chen et al., 2008).

Vagal neurocircuits have a well-described role in nausea and vomiting (see Babic and Browning, 2014) and the role of vagal afferent fibers in emesis have been most extensively studied in the context of chemotherapy-induced nausea and vomiting (CINV) or postoperative nausea and vomiting (PONV). Several chemotherapy agents induce the release of 5-HT from EC cells which activates 5-HT receptor on vagal afferent terminals (Endo et al., 1990, 2000; Horn et al., 2004); vagotomy decreases emesis induced by cytotoxic drugs while 5-HT receptor selective antagonists are particularly efficacious clinically in preventing CINV and PONV (Hawthorn et al., 1988; Andrews et al., 1990; Endo et al., 2000; Darmani and Johnson, 2004; Andrews and Horn, 2006). The presumed site of action of these 5-HT receptor selective antagonists is at peripheral vagal afferent terminals (Endo et al., 2000) although it should be noted that centrally applied 5-HT receptor antagonists also attenuate CINV, suggesting actions at brainstem 5-HT receptors (Leslie et al., 1990; Reynolds et al., 1991; Liu et al., 2003; Darmani and Ray, 2009) while 5-HT-induced disruptions in normal GI motility patterns may also contribute to CINV and PONV (Endo et al., 2000; Glatzle et al., 2002; Tonini, 2005). Similarly, the nausea and vomiting associated with several infectious agents, including rotavirus (Hagbom et al., 2011), Salmonella typhimurium (Jensen et al., 1997), and campylobacter (Blakelock and Beasley, 2003) has also been associated with the activation of vagal afferent 5-HT receptors subsequent to intestinal 5-HT release.

The role of vagal afferent 5-HT receptors in various forms of visceral hypersensitivity and nociceptive processing has been the focus of considerable attention from several groups although there are conflicting reports as to the extent of the involvement of vagal, rather than spinal, pathways. Several studies have suggested that vagal afferent fibers, and vagal afferent 5-HT receptors in particular, are important in the inhibitory modulation of spinal nociceptive transmission. Briefly, when administered intravenously, 5-HT induces a dose-dependent inhibition of the tail flick reflex, and this anti-nociceptive effect is dependent upon intact vagal pathways since it is abolished by either cervical vagotomy, nodose ganglionectomy, or neonatal capsaicin pretreatment (Meller et al., 1992). In a similar manner, vagotomized rats display an enhanced visceromotor response to colorectal distention (alldynia), effects that are lost following application of the local anesthetic lidocaine to the abdominal vagus (Chen et al., 2008). The specific involvement of 5-HT receptors in these responses was confirmed by studies investigating stress-induced visceral hyperalgesia, which demonstrated that subcutaneous administration of 5-HT receptor selective antagonists increased the visceromotor response to colorectal distension, actions that were prevented by perivagal capsaicin (Bradesi et al., 2007, NB—it should be noted that perivagal capsaicin does not produce a selective vagal deafferentation but also causes a significant degree of damage to vagal efferent motoneurons, Browning et al., 2013a). Thus, it appears that 5-HT receptor-dependent activation of vagal afferents inhibits the noxious stimulation of spinal afferents although the central nuclei responsible for this descending modulation have not been defined fully (Ren et al., 1990; Randich and Gebhart, 1992).

Such an anti-nociceptive role of vagal afferent 5-HT receptors appears consistent across several visceral hypersensitivity models suggesting common mechanistic pathophysiology. In experimental models of duodenal acidification-induced gastric hypersensitivity, for example, intestinal acidification enhances the pressor response observed in response to gastric distention; this pressor response is enhanced by 5-HT receptor selective agonists (Nakata-Fukuda et al., 2014) while administration of 5-HT receptor selective antagonists inhibits the sensitization to distention that occurs in humans (Vanuytsel et al., 2011).

Activation of vagal afferent 5-HT receptors also have well described roles in the immune responses elicited by antigen challenge in sensitized animal models, where 5-HT released following mast cell degranulation activates vagal afferents to modulate the visceral hypersensitivity and motor response to the immune challenge (Castex et al., 1995; Jiang et al., 2000; Chen et al., 2009). It should be noted, however, that other studies have suggested that the principle action of the sensory vagus in these antigen challenged models may be to monitor GI activity during the anaphylactic response, rather than playing a critical role in symptom generation (Scott et al., 1998). In this regard, studies have noted that the role of vagal afferents to inhibit nociceptive signaling may have temporally restricted actions, triggering endogenous antinociception at the early stages of allergen challenge and thereafter declining over time (Chen et al., 2009).

In part, this time-dependent decline in response may be related to the functional presence and activity of 5-HT receptors on vagal afferents; prolonged activation of 5-HT receptors leads to receptor desensitization and internalization (Freeman et al., 2006) and a decrease in receptor mRNA levels has been observed following chronic immune challenge (Chen et al., 2009). Also of relevance in this regard are the altered expression levels of serotonin transporters, particularly the serotonin-selective reuptake transporter (SERT) in several visceral hypersensitivity disorders. 5-HT signaling is terminated by reuptake into intestinal epithelium or nerve terminals via specialized transporter systems; alterations in SERT levels, therefore, are critical in regulating the availability, activity and duration of 5-HT signaling. SERT expression is downregulated in several hypersensitivity disorders including intestinal inflammatory conditions such as IBD as well as some, but not all, patients with diarrhea-predominant IBS (Coates et al., 2004; Camilleri et al., 2007; Foley et al., 2011). It is unclear whether such alterations in SERT contribute to dysregulated vagal afferent signaling in these groups, however. It is also unclear whether SERT expression levels are altered centrally in response to visceral hypersensitivity disorders; blocking SERT activity in the brainstem decreases glutamatergic synaptic transmission from the central terminals of vagal afferents due to the activation of
presynaptic 5-HT_{1A} receptors, the activity of which are more tightly regulated by physical proximity to uptake transporters (Hosford et al., 2014). An increase in brainstem 5-HT levels in response to altered SERT activity may, therefore, have the potential to dramatically alter the gain of GI vagal afferent information transfer.

Many chronic pain syndromes, including IBS, are significantly more prevalent in women suggesting a role for gonadal hormones in the modulation of visceral sensitivity (Mulak et al., 2014). Estradiol has been shown to increase the secretion of 5-HT from intestinal mucosal mast cells in rats (Yan et al., 2014) causing the activation of vagal afferent 5-HT_{3} receptors and an inhibition of the visceromotor response to colorectal distention in rats. It should also be noted, however, that estradiol has pronociceptive actions via spinal mechanisms; an imbalance between vagal antinociceptive and spinal pronociceptive pathways as estrogen levels fluctuate during the menstrual cycle may potentially exacerbate visceral sensitivity in susceptible IBS females (Yan et al., 2014).

Although, the regulation of food intake and energy homeostasis is generally considered to involve the integration of "higher" CNS centers with autonomic nuclei, the role of vago-vagal neurocircuits in the regulation of early satiety signaling has been the subject of renewed attention by several laboratory groups (Page et al., 2012; Dockray, 2013; de Lartigue, 2014; Kentish and Page, 2014). Diet-induced obesity is known to compromise the excitability and responsiveness of GI vagal afferent fibers (Covasa et al., 2000a,b; Swartz et al., 2010; Kentish et al., 2012) and neurons (Donovan et al., 2007; Paulino et al., 2009; Daly et al., 2011; de Lartigue et al., 2011). The mechanism responsible for this attenuated excitability has not been elucidated fully although studies in both obese mice and rats demonstrating a decreased membrane input resistance and increased membrane capacitance are suggestive of an increase in resting background potassium conductance(s) (Daly et al., 2011; Browning et al., 2013b). Studies have suggested that 5-HT_{3} receptor expression is downregulated following short term exposure to a high fat diet (Nefti et al., 2009) and 5-HT_{3}-dependent activation of vagal afferent fibers is attenuated in diet-induced obese mice (Daly et al., 2011) but it is unclear whether this reflects the obesity-induced generalized decrease in vagal afferent excitability or a more specific decline in 5-HT_{3} function. In our recent studies in pre-obese rats fed a high fat diet, however, we have not observed an attenuated or compromised response of gastric vagal afferent fibers to 5-HT_{3} receptor activation (Troy et al., 2015), suggesting that obesity itself, rather than exposure to a high fat diet, may be responsible for the compromised 5-HT_{3} receptor signaling.

Evidence from several fields have suggested that vagal neurocircuits are not static relay networks where afferent activation triggers formulaic and unmodulated output responses. Rather, vagal neurocircuits display a remarkable degree of plasticity with their excitability and responsiveness being modulated readily by diet, insult or injury (Browning and Travagli, 2001, 2011; Bielefeldt et al., 2002a,b; Kollarik and Undem, 2002; Dang et al., 2004; Kang et al., 2004, 2005; Tolstykh et al., 2004; Hermans et al., 2008; Kentish et al., 2012, 2014; Browning et al., 2013b). In this regard, it is interesting to note that allergic challenge in sensitized animals induces a 5-HT_{3}-dependent exposure of tachykinin receptor responses in respiratory vagal afferents and neurons (Weinreich et al., 1997; Moore et al., 1999, 2000, 2002); similar changes in GI afferents and neurons may also play a role in visceral hypersensitivity. Also of relevance is the finding that, despite being asymptic, cross-talk exists between nodose ganglion neurons, where excitation of one neuron may influence that of a neighboring neuron by neurotransmitter-dependent and -independent means (Oh and Weinreich, 2002). The nodose ganglion (or nodose-jugular complex) houses the cell bodies of all vagal afferent neurons; although a generalized viscerotopic organization of soma has been proposed with neurons innervating the esophagus and aortic depressor nerve being located more rostrally with neurons innervating the stomach and pancreas being located more caudally (Zhuo et al., 1997), clearly cross-talk between neurons, may provide a means by which neurons innervating different visceral organs, or different GI areas, may influence or modulate activity of unrelated neurons.

**CONCLUSIONS**

5-HT and 5-HT_{3} receptors in particular, are clearly important in gut-brain signaling and in the regulation and modulation of several vagally-mediated GI physiological reflexes and may play additional roles in several pathophysiological conditions. 5-HT_{3} receptors also appear open to modulation; extracellular glucose levels, for example, traffic 5-HT receptors to and from the neuronal membrane of GI nodose neurons amplifying or attenuating the 5-HT-induced response, while some, but not all, reports suggest alterations in receptor function by diet induced obesity. It would be surprising, however, if dietary micronutrients were the only mediators 5-HT receptor plasticity. Antigen challenge, for example, has been shown to induce 5-HT_{3} receptor dependent unmasking of tachykinin functions in respiratory nodose neurons; future studies investigating whether similar changes occur in GI nodose neurons may provide novel treatment strategies for allergen induced visceral hypersensitivity. Also intriguing is the apparent dichotomy between vagal afferent 5-HT_{3} responses; excessive activation of vagal afferent 5-HT_{3} receptors induces nausea and vomiting whereas several reports suggest an initial, temporally discrete anti-nociceptive response in stress-induced hypersensitivity. These (and other) diverse 5-HT_{3} receptor-dependent responses present obvious problems to the therapeutic use of receptor selective agonists or antagonists yet their more readily accessible nature means that vagal afferent 5-HT_{3} receptors still present an attract target for translational research.

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REFERENCES

Andresen, M. C., and Kunze, D. L. (1994). Nucleus tractus solitarius–
gateway to neural circulatory control. Annu. Rev. Physiol. 56, 93–116. doi: 10.1146/annurev.ph.56.030194.000521

Andrews, P. L., Davis, C. J., Bingham, S. D., Davidson, H. L., Hawthorn, J., and Maskell, L. (1990). The abdominal visceral innervation and the emetic reflex: pathways, pharmacology, and plasticity. Can. J. Physiol. Pharmacol. 68, 325–345. doi: 10.1139/y90-047

Andrews, P. L., and Horn, C. C. (2006). Signals for nausea and emesis: implications for models of upper gastrointestinal diseases. Auton. Neurosci. 120, 100–115. doi: 10.1016/j.autneu.2006.01.008

Babic, T., and Browning, K. N. (2014). The role of vagal neurocircuits in the regulation of nausea and vomiting. Eur. J. Pharmacol. 722, 38–47. doi: 10.1016/j.ejphar.2013.08.047

Bacic, T., Troy, A. E., Fortna, S. R., and Browning, K. N. (2012). Glucose-
dependent trafficking of 5-HT(3) receptors in rat gastrointestinal afferent neurons. Neurogastroenterol. Motil. 24, e476–e488. doi: 10.1111/j.1365-2982.2012.01987.x

Baptista, V., Browning, K. N., and Travagli, R. A. (2007). Effects of 5-hydroxytryptamine and 5-hydroxytryptophan on peristalsis; the local production of 5-HT and its release in relation to intraluminal pressure and propulsive activity. J. Physiol. 140, 381–407.

Callera, J. C., Sevoz, C., Laguzzi, R., and Machado, B. H. (1997). Microinjection of a serotonin3 receptor agonist into the NTS of unanesthetized rats inhibits the bradycardia evoked by activation of the baro- and chemoreflexes. J. Auton. Nerv. Syst. 63, 127–136. doi: 10.1016/S0165-1838(96)00140-3

Camilleri, M., Andrews, C. N., Bharucha, A. E., Carlson, P. J., Ferber, L., Stephens, D., et al. (2007). Alterations in expression of p11 and SERT in mucosal biopsy specimens of patients with irritable bowel syndrome. Gastroenterology 132, 17–25. doi: 10.1053/j.gastro.2006.11.020

Castex, N., Fioramonti, J., Fargas, M. J., and Bueno, L. (1995). c-fos expression in specific rat brain nuclei after intestinal anaphylaxis: involvement of 5-HT3 receptors and vagal afferent fibers. Brain Res. 688, 149–160. doi: 10.1016/0006-8993(95)00526-V

Chen, S. L., Ji, J., Zhang, L., Dong, X., Gao, W., Mo, J., et al. (2009). 5-
HT3 receptors mediate the time-dependent vagal afferent modulation of nociception during chronic food allergen-sensitized visceral hyperalgesia in rats. Neurogastroenterol. Motil. 21, 1222–1213. doi: 10.1111/j.1365-2982.2009.01335.x

Chen, S. L., Wu, X. Y., Cao, Z. J., Fan, J., Wang, M., Owyang, C., et al. (2008). Subdiaphragmatic vagal afferent nerves mediate visceral pain. Am. J. Physiol. Gastrointest. Liver Physiol. 294, G1441–G1449. doi: 10.1152/ajpgi.00588.2007

Coates, M. D., Mahoney, C. R., Lindem, D. R., Sampson, J. E., Chen, J., Blaszyk, H., et al. (2004). Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. Gastroenterology 126, 1657–1664. doi: 10.1053/gast.2004.03.013

Dekom, N. S., Marciani, L., Blackshaw, E., Wright, J., Parker, M., Yano, T., et al. (2003). Effect of a novel 5-HT(3) receptor agonist MKC-733 on upper gastrointestinal motility in humans. Aliment. Pharmacol. Ther. 18, 1039–1048. doi: 10.1046/j.1365-2036.2003.01797.x

Cottrell, G. T., and Ferguson, A. V. (2004). Sensory circumventricular organs: central roles in integrated autonomic regulation. Regul. Pept. 117, 11–23. doi: 10.1016/j.regpep.2003.09.004

Covasa, M., Grahn, J., and Ritter, R. C. (2000a). High fat maintenance diet attenuates hindbrain neural response to CCK. Regul. Pept. 86, 83–88. doi: 10.1016/S0167-0115(99)00084-1

Covasa, M., Grahn, J., and Ritter, R. C. (2000b). Reduced hindbrain and enteric neural response to intestinal oleate in rats maintained on high-fat diet. Auton. Neurosci. 84, 8–18. doi: 10.1016/S1566-0702(00)00176-4

Darmani, N. A., and Johnson, J. C. (2004). Central and peripheral mechanisms contribute to the antiemetic actions of delta-9-tetrahydrocannabinol against 5-hydroxytryptophan-induced emesis. Eur. J. Pharmacol. 488, 201–212. doi: 10.1016/j.ejphar.2004.02.018

Darmani, N. A., and Ray, A. P. (2009). Evidence for a re-evaluation of the neurochemical and anatomical bases of chemotherapy-induced vomiting. Chem. Rev. 109, 3158–3199. doi: 10.1021/cr900117p

d'Artigue, L. (2014). Putative roles of neuropeptides in vagal afferent signaling. Physiol. Behav. 136, 155–169. doi: 10.1016/j.physbeh.2014.03.011

d'Artigue, L., de La Serre, C. B., and Raybould, H. E. (2011). Vagal afferent neurons in high fat diet-induced obesity; intestinal microflora, gut inflammation and cholecystokinin. Physiol. Behav. 105, 100–105. doi: 10.1016/j.physbeh.2011.02.040

Decker, V., Surprenant, A., and North, R. A. (1989). 5-HT(3) receptors are membrane ion channels. Nature 339, 706–709. doi: 10.1038/339706a0
Browning

Vagal afferent 5-HT$_3$ receptors

Lacolley, P., Owen, J. R., Sandock, K., Lewis, T. H., Bates, J. N., Robertson, T. P., et al. (2006b). Occipital artery injections of 5-HT may directly activate the cell bodies of vagal and glossopharyngeal afferent cell bodies in the rat. *Neuroscience* 143, 289–308. doi: 10.1016/j.neuroscience.2006.08.047

Leal-Cardoso, H., Koschorke, G. M., Taylor, G. E., and Weinreich, D. (1993). Electrophysiological properties and chemosensitivity of acutely isolated nodose ganglion neurons of the rabbit. *JANS* 45, 29–39. doi: 10.1016/0165-1838(93)90359-3

Leslie, R. A., Reynolds, D. J. M., Andrews, P. L., Grahame-Smith, D. G., Davis, C. J., and Harvey, J. M. (1990). Evidence for presynaptic 5-hydroxytryptamine 3 recognition sites on vagal afferent terminals in the brainstem of the ferret. *Neuroscience* 38, 667–673. doi: 10.1016/0306-4522(90)90060-H

Liu, Y., Hamaue, N., Endo, T., Hirafuji, M., and Minami, M. (2003). 5-hydroxytryptamine (5-HT) concentrations in the hippocampus, the hypothalamus and the medulla oblongata related to cisplatin-induced pica of rats. *Res. Commun. Mol. Pathol. Pharmacol.* 113, 94–117. doi: 10.1111/j.1365-2840.2003.tb00277.x

MacGregor, I. L., Gueller, R., Watts, H. D., and Meyer, J. H. (1976). The effect of acute hyperglycaemia on gastric emptying in man. *Gastroenterology* 70, 190–196.

Mawe, G. M., and Hoffmann, J. M. (2013). Serotonin signalling in the gut–functions, dysfunctions and therapeutic targets. *Nat. Rev. Gastroenterol. Hepatol.* 10, 473–486. doi: 10.1038/nrgastro.2013.105

Mazda, T., Yamamoto, H., Fujimura, M., and Fujimya, M. (2004). Gastric distension-induced release of 5-HT stimulates c-fos expression in specific brain nuclei via 5-HT$_3$ receptors in conscious rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* 287, G228–G235. doi: 10.1152/ajpgi.00373.2003

Mei, N. (1978). Vagal glucoreceptors in the small intestine of the cat. *J. Physiol.* 282, 485–506. doi: 10.1113/jphysiol.1978.sp012477

Meller, S. T., Lewis, S. J., Brody, M. J., and Gebhart, G. F. (1992). Vagal afferent-mediated inhibition of a nociceptive reflex by i.v. serotonin in the rat. II. Role of 5-HT receptor subtypes. *Brain Res.* 585, 71–86. doi: 10.1016/0006-8993(92)91192-H

Moore, K. A., Oh, E. J., and Weinreich, D. (2002). 5-HT$_3$(3) receptors mediate inflammation-induced unmasking of functional tachykinin responses in vitro. *J. Appl. Physiol.* 92, 2529–2534. doi: 10.1152/japplphysiol.00974.2001

Moore, K. A., Taylor, G. E., and Weinreich, D. (1999). Serotonin unmasks functional NK-2 receptors in vagal sensory neurons of the guinea pig. *J. Physiol.* 514, 111–124. doi: 10.1111/j.1469-7793.1999.11111.x

Moore, K. A., Undem, B. J., and Weinreich, D. (2000). Antigen inhaled unmasks NK-2 tachykinin receptor-mediated responses in vagal afferents. *Am. J. Resp. Crit. Care Med.* 161, 232–236. doi: 10.1164/ajrccm.161.1.9903091

Mulak, A., Taché, Y., and Larache, M. (2014). Sex hormones in the modulation of irritable bowel syndrome. *World J. Gastroenterol.* 20, 2433–2448. doi: 10.3748/wjg.v20.i17.2433

Mussa, B. M., Sartor, D. M., and Verberne, A. J. (2008). Activation of cholecystokinin (CCK1) and serotonin (5-HT3) receptors increases the discharge of pancreatic vagal afferents. *Eur. J. Pharmacol.* 601, 198–206. doi: 10.1016/j.ejphар.2008.11.007

Mussa, B. M., Sartor, D. M., and Verberne, A. J. (2010). Dorsal vagal preganglionic neurons: differential responses to CCK1 and 5-HT$_3$ receptor stimulation. *Auton. Neurosci.* 156, 36–43. doi: 10.1016/j.autneu.2010.03.001

Nakata-Fukuda, M., Hirata, T., Kato, Y., Yamano, M., Yokoyama, T., and Uchiyama, Y. (2014). Inhibitory effect of the selective serotonin 5-HT(3) receptor antagonist ramsoetron on duodenal acidification-induced gastric hypersensitivity in rats. *Eur. J. Pharmcol.* 731, 88–92. doi: 10.1016/j.ejphar.2014.02.040

Nefiti, W., Chaumontet, C., Fromentin, G., Tomé, D., and Darcel, N. (2009). A high-fat diet attenuates the central response to within-meal satiation signals and modifies the receptor expression of vagal afferents in mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R1681–R1686. doi: 10.1152/ajpregu.90733.2008

Nosjean, A., Compoint, C., Buissere-Delmas, C., Ozer, H. S., Merahi, N., Puizilout, J. J., et al. (1990). Serotonergic projections from the nodose ganglia to the nucleus tractus solitarius: an immunohistochemical and double labeling study in the rat. *Neurosci. Lett.* 114, 22–26. doi: 10.1016/0304-3940(90)00226-6

Oh, E. J., and Weinreich, D. (2002). Chemical communication between vagal afferent somata in nodose Ganglia of the rat and the Guinea pig in vitro. *J. Neurophysiol.* 87, 2801–2807.
Vanuytsel, T., Karamanolis, G., Van, O. L., Vos, R., and Tack, J. (2011). Influence of carbohydrate and other gut luminal factors on excitatory synaptic transmission in the nucleus tractus solitarius of the rat. *Eur. J. Pharmacol.* 671, 45–52. doi: 10.1016/j.ejphar.2011.09.164

Thor, K. B., and Helke, C. J. (1987). Serotonin- and substance P-containing projections to the nucleus tractus solitarii of the rat. *J. Comp. Neurol.* 265, 275–293. doi: 10.1002/cne.902650210

Thor, K. B., and Helke, C. J. (1989). Serotonin and substance P colocalization in medullary projections to the nucleus tractus solitarius: dual-colour immunohistochemistry combined with retrograde tracing. *J. Chem. Neuroanat.* 2, 139–148.

Thor, K. B., Hill, K. M., Harrod, C., and Helke, C. J. (1988). Immunohistochemical and biochemical analysis of serotonin and substance P colocalization in the nucleus tractus solitarii and associated afferent ganglia of the rat. *Synaps* 2, 225–231. doi: 10.1002/syn.890020309

Tolstykh, G., Belugin, S., and Mifflin, S. (2004). Responses to GABA(A) receptor activation are altered in NTS neurons isolated from chronic hypoxic rats. *Brain Res.* 1006, 107–113. doi: 10.1016/j.brainres.2004.01.060

Tonini, M. (2005). 5-Hydroxytryptamine effects in the gut: the 3, 4, and 7 receptors. *Neurogastroenterol. Motil.* 17, 637–642. doi: 10.1111/j.1365-2982.2005.00716.x

Travagli, R. A., Hermann, G. E., Browning, K. N., and Rogers, R. C. (2006). Brainstem circuits regulating gastric function. *Annu. Rev. Physiol.* 68, 279–305. doi: 10.1146/annurev.physiol.68.040504.094635

Troy, A. E., Simmonds, S. S., Stocker, S. D., and Browning, K. N. (2015). High fat diet attenuates glucose-dependent facilitation of 5-HT3 mediated responses in rat gastric vagal afferents. *J. Physiol.* doi: 10.1113/JP271558. [Epub ahead of print].

Tuladhar, B. R., Kaisar, M., and Naylor, R. J. (1997). Evidence for a 5-HT3 receptor involvement in the facilitation of peristalsis on mucosal application of 5-HT in the guinea pig isolated ileum. *Br. J. Pharmacol.* 122, 1174–1178. doi: 10.1038/sj.bjp.0701503

Vanuytsel, T., Karamanolis, G., Van, O. L., Vos, R., and Tack, J. (2011). Influence of ondansetron on gastric sensorimotor responses to short duodenal acid infusion in healthy volunteers. *Neurogastroenterol. Motil.* 23, 226–232, e115. doi: 10.1111/j.1365-2982.2010.01631.x

Wan, S., and Browning, K. N. (2008b). Glucose increases synaptic transmission from vagal afferent central nerve terminals via modulation of 5HT3 receptors. *Am. J. Physiol. Gastrointest. Liver Physiol.* 294, G757–G763. doi: 10.1152/ajpgi.00576.2007

Wan, S., and Browning, K. N. (2008a). D-Glucose modulates synaptic transmission from the central terminals of vagal afferent fibers. *Am. J. Physiol. Gastrointest. Liver Physiol.* 294, G757–G763. doi: 10.1152/ajpgi.00576.2007

Wang, Y., Ramage, A. G., and Jordan, D. (1996). Mediation by 5-HT3 receptors of an excitatory effect of 5-HT on dorsal vagal preganglionic neurons in anesthetized rats: an ionophoretic study. *Br. J. Pharmacol.* 118, 1697–1704. doi: 10.1111/j.1476-5381.1996.tb15594.x

Weihe, D., Moore, K. A., and Taylor, G. E. (1997). Allergic inflammation in isolated vagal sensory ganglia unmasks silent NK-2 tachykinin receptors. *J. Neurosci.* 17, 7683–7693.

Wu, Q., Clark, M. S., and Palmiter, R. D. (2012). Deciphering a neuronal circuit that mediates appetite. *Nature* 483, 594–597. doi: 10.1038/nature10899

Wu, X. Y., Zhou, J. X., Gao, J., Owyang, C., and Li, Y. (2005). Neurochemical phenotype of vagal afferent neurons activated to express C-FOS in response to luminal stimulation in the rat. *Neuroscience* 130, 757–767. doi: 10.1016/j.neuroscience.2004.09.060

Yan, X. J., Feng, C. C., Liu, Q., Zhang, L. Y., Dong, X., Liu, Z. L., et al. (2014). Vagal afferents mediate antinociception of estrogen in a rat model of visceral pain: the involvement of intestinal mucosal mast cells and 5-hydroxytryptamine 3 signaling. *J. Pain* 15, 204–217. doi: 10.1016/j.jpain.2013.10.012

Zhou, X., and Galligan, J. J. (1999). Synthetic activation and properties of 5-hydroxytryptamine(3) receptors in myenteric neurons of guinea pig intestine. *J. Pharmacol. Exp. Ther.* 290, 803–810.

Zhu, J. X., Wu, X. Y., Owyang, C., and Li, Y. (2001). Intestinal serotonin acts as a paracrine substance to mediate vagal signal transmission evoked by luminal factors in the rat. *J. Physiol.* 530, 431–442. doi: 10.1111/j.1469-7793.2001.0431.x

Zhuo, H., Ichikawa, H., and Helke, C. J. (1997). Neurochemistry of the nodose ganglion. *Prog. Neurobiol.* 52, 79–107. doi: 10.1006/syn.890020309

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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