Differential organization of excitatory and inhibitory synapses within the rat dorsal vagal complex

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Running title: GABA and glutamate inputs to DMV neurons

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ABSTRACT

The dorsal motor nucleus of the vagus (DMV) is pivotal in the regulation of upper gastrointestinal functions, including motility and both gastric and pancreatic secretion. DMV neurons receive robust GABA- and glutamatergic inputs. Microinjection of the GABA$_A$ antagonist bicuculline (BIC) into the DMV increases pancreatic secretion and gastric motility whereas the glutamatergic antagonist kynurenic acid (KYN) is ineffective unless preceded by microinjection of BIC.

We used whole cell patch clamp recordings with the aim of unveiling the brainstem neurocircuitry that uses tonic GABA- and glutamatergic synapses to control the activity of DMV neurons in a brainstem slice preparation.

Perfusion with BIC altered the firing frequency of 71% of DMV neurons, increasing firing frequency in 80% of the responsive neurons and decreasing firing frequency in 20%. Addition of KYN to the perfusate either decreased (52%) or increased (25%) the firing frequency of BIC-sensitive neurons.

When KYN was applied first, the firing rate was decreased in 43% and increased in 21% of the neurons; further perfusion with BIC had no additional effect in the majority of neurons.

Our results indicate that there are several permutations in the arrangements of GABA- and glutamatergic inputs controlling the activity of DMV neurons. Our data support the concept of brainstem neuronal circuitry that may be wired in a finely tuned, organ- or function-specific manner that permits precise and discrete modulation of the vagal motor output to the GI tract.
INTRODUCTION

Sensory information from the gastrointestinal (GI) tract is encoded by chemo- and mechanoreceptors with different modalities, responses and properties (reviewed in (11)). Regardless of their function(s) or modalities, however, primarily non-noxious visceral sensory information is transmitted through the afferent vagus into the brainstem via a glutamatergic synapse at the level of the nucleus tractus solitarius (NTS) (2; 3; 8; 54). The NTS comprises various subnuclei that appear to be organized viscerotopically (1; 9; 57), such that the vagal sensory input from each visceral organ is concentrated in a particular subnucleus. The subnucleus centralis (cNTS), for example, receives inputs almost exclusively from the esophagus.

The motor output to the GI tract is provided by the spontaneously firing neurons of the dorsal motor nucleus of the vagus (DMV), which, in contrast to NTS neurons, are organized in neuronal “columns” that project to the viscera through each of the five subdiaphragmatic vagal branches (reviewed in (57)). DMV neurons within each “column”, however, are not identical but comprise heterogeneous neuronal populations that differ in their neurochemical, morphological and electrophysiological properties (13; 16; 30; 34; 37; 40; 55).

The vagal output to the GI tract is involved in the regulation of several different functions related to digestive processes, which include reflex control of gastrointestinal motility, gastric, pancreatic and duodenal secretion, as well as homeostatic adaptations to external stimuli and challenges. All of these functions require a fine modulatory control that is achieved by the vast array of afferent inputs from multiple regions of the CNS, which monitor the physiological state of the animal and convey this information to the brainstem nuclei. NTS and DMV neurons, as well as their synaptic connections, represent the final CNS locus for the integrated modulation of the sensory-motor brain-gut axis to synchronise the activity of the GI tract.

NTS and DMV neurons are connected through a dense synaptic network that uses mainly GABA, glutamate and catecholamines as neurotransmitters (23; 31; 49; 56; 59). In recent years many in vitro studies have reported the modulation of these synapses by various substances (14; 15; 18; 21; 24; 25; 28; 33; 58). A large array of in vivo studies have also correlated the effects of microinjections of neurotransmitters and neuromodulators in the dorsal vagal complex (DVC; i.e. DMV, NTS and area postrema) with responses in the various GI organs. More specifically, a series of studies focused on the GI responses to microinjections of the GABA and glutamate-related substances in the brainstem. Microinjections of the specific GABA<sub>A</sub> antagonist bicuculline (BIC) into the DMV increase gastric acid secretion, intragastric...
pressure and exocrine pancreatic secretions (27; 44; 53), suggesting that DMV neurons innervating the stomach and the exocrine pancreas receive tonic GABAergic inputs. Glutamate, in contrast, appears to exert site-specific effects on esophageal and gastric motility. Activation of rostral DMV neurons by glutamate microinjection elicits gastric contractions and increases tone of the lower esophageal sphincter, whereas glutamate microinjections in the caudal DMV have the opposite effect (45; 50; 61). Moreover, microinjections of the specific ionotropic glutamate antagonist kynurenic acid (KYN) alone have no effect on gastric motility, but abolish the effect of BIC (53). These observations suggest that tonically active GABAergic synapses exert their effect upon gastric motility via actions involving the activation of glutamate synapses. Taken together, it is evident that GABA- and glutamatergic inputs to DMV neurons play a fundamental role in the regulation of vagal output; their role in the modulation of the firing rate of DMV neurons, however, has not been investigated yet.

This study used a brainstem slice preparation to investigate whether 1) the firing rate of DMV neurons is modulated by tonic GABAergic and glutamatergic inputs; and, 2) all the GABA- and glutamatergic inputs onto DMV neurons induce similar responses.

MATERIALS AND METHODS

All experiments were conducted with the approval of the IACUC committee and according to NIH regulations.

Retrograde Tracing

A subgroup of Sprague-Dawley rats (14 days old) of both sexes were anesthetized with a solution of isoflurane with air (400-600 ml/min). Once a suitable depth of anesthesia was obtained (checked via the foot pinch withdrawal reflex), the abdominal and thoracic areas were shaved and cleaned with disinfectant and a laparotomy was performed. Crystals of the fluorescent tracer 1,1'-dioctadecyl-3,3,3',3'-tetra-methyl-indo-carbocyanine perchlorate (DiI; Molecular Probes, Eugene, OR) were applied to either the major curvature of the gastric fundus or corpus. In order to confine the dye to the gastric areas of interest, the application site was embedded in a fast-hardening epoxy compound. The epoxy compound was allowed to dry, and
the area was examined visually to ensure that the dye had remained affixed to the organ surface and not made contact with other tissues. The surgical area was washed with warm sterile saline solution, blotted with cotton tips and the laparotomy was closed. The animals were allowed to recover for at least seven days.

**Electrophysiology**

The brainstems were removed as described previously (15; 16). Briefly, rats were anesthetized with isofluorane and euthanized via administration of bilateral pneumothorax prior to removal of the brainstem, which was then placed in oxygenated Krebs’ solution at 4°C. Five to 6 coronal sections (300 μm thick) through the dorsal vagal complex (DVC) were cut on a vibratome and incubated in oxygenated Krebs’ solution at 30°C for at least 90 min. A single slice was transferred to a custom-made perfusion chamber (volume 500 µl) and kept in place with a nylon mesh. The chamber was maintained at 32 ± 1°C by perfusion with warmed, oxygenated Krebs’ solution at a rate of 2.5-3.0 ml/min.

In the subgroup of rats that underwent placement of DiI on the corpus or fundus, prior to electrophysiological recording, DiI-labelled neurons were identified by using a Nikon E600-FN microscope equipped with tetramethylrhodamine isothiocyanate epifluorescent filters. Once labeled neurons were identified, whole cell recordings were made under bright-field illumination using DIC (Nomarski) optics.

Whole cell recordings were made with patch pipettes (2-5MΩ tip resistance) filled with a potassium gluconate solution using an Axopatch 2B amplifier (Molecular Devices, Sunnyvale, CA, USA). Recordings of non-labelled DMV neurons were conducted only in the medial portions of the nucleus, i.e. the area which comprises exclusively gastric projecting DMV neurons (29; 46; 52) as well as from gastric-projecting DMV neurons labeled unequivocally with Dil. Data were filtered at 2 kHz, digitized via a Digidata 1320A interface (Molecular Devices), acquired, stored and analyzed on a personal computer utilizing pClamp9 software (Molecular Devices). Recordings were accepted only if the series resistance was <20 MΩ. The neuronal firing rate was set to approximately 1 pulse/s via injection of direct current (DC).

Bicuculline methiodide (BIC; 50μM; Sigma Aldrich, St. Louis, MO) and kynurenic acid (KYN; 1mM; Sigma Aldrich, St. Louis, MO) were applied to the bath via a series of manually operated valves. Following a one minute baseline period, each antagonist, i.e. BIC or KYN, was applied for 7min in random order. After 7min, the second drug, i.e. KYN or BIC, was added to the perfusate for a further 7min, after which all drugs were washed out until the firing rate
recovered toward baseline values, usually up to 20 minutes. In one group of cells, the GABA\(_A\) receptor antagonist SR95531 (gabazine; 20\(\mu\)M; Sigma Aldrich, St. Louis, MO) was applied instead of BIC. To assess the effects of drugs, each cell served as its own control. Only one cell per slice was tested with each drug. Data were analyzed by averaging the number of action potentials in control conditions, during drug perfusion and wash-out periods.

**Statistical analysis**

Data were analyzed by comparing action potential frequency at baseline and during treatment by using paired student’s t-test, intergroup comparisons were analysed with the \(\chi^2\) test. Significance was defined as \(p<0.05\).

**RESULTS**

Throughout the manuscript we will use a simplified graphic representation of the NTS-DMV synaptic connections as depicted in Figure 1 as a template for the potential synaptic circuit. In each subsequent figure, the putative site of action of the drug tested is highlighted. Since the slice was perfused with specific antagonists, a variation in the neuronal firing rate indicates a site of action on a synapse that is tonically active.

**Bicuculline (BIC) modulates the firing rate of the majority of DMV neurons**

Effects of 50 \(\mu\)M BIC on the firing rate were tested in 62 DMV neurons. In these neurons, the basal firing rate was 1.03 \(\pm\) 0.03 action potentials/s. Perfusion of the slice with BIC altered the firing rate in 44 of the 62 (i.e. 71%) neurons tested, suggesting that the vast majority of DMV neurons receive a tonic GABAergic input. In the remaining 18 (i.e. 29%) neurons, perfusion with BIC did not alter their firing rate, indicating that this neuronal subpopulation is not under a tonic GABAergic influence. In one group of cells, the GABA\(_A\) receptor antagonist SR95531, which inhibits phasic, but not tonic GABA currents (4; 31) was applied instead of BIC. Of the 22 cells tested, 5 (23 %) increased their firing rate from 1.0 \(\pm\) 0 action potentials/s to 1.8 \(\pm\) 0.25 action potentials/s, 8 (36 %) decreased their firing rate from 1.0 \(\pm\) 0 to 0.18 \(\pm\) 0.07 action potentials/s and the remaining 9 (41 %) did not respond to SR 95531. Since a greater
percentage of neurons responded to BIC than to SR 95531, and since BIC blocks both tonic and phasic GABAergic currents (4; 31), we used BIC for experiments in which effects of KYN were tested.

BIC increased the firing rate from $1.02 \pm 0.04$ to $3.13 \pm 0.52$ action potentials/s in 35 of the 44 (i.e. 80%) responsive neurons (figure 2A,B). In these neurons, perfusion with 1mM KYN in the continuous presence of BIC either decreased (from $4.8 \pm 0.25$ to $1.67 \pm 0.06$ action potentials/s; N=16) or blocked ($0 \pm 0$ action potentials/s; N=3) the firing rate in 19 (i.e. 54%; figure 2A,C) and increased the firing rate in 10 (i.e. 29%; from $2.29 \pm 0.27$ to $3.1 \pm 0.3$ action potentials/s; figure 2D) neurons. The firing rate of the remaining 6 (i.e. 17%) neurons was unaffected by KYN perfusion (from $2.0 \pm 0.40$ to $2 \pm 0.38$ action potentials/s). Data are summarized in Table 1 and the potential sites of action of BIC and KYN are depicted in figure 2B-D.

These data suggest that the majority of DMV neurons receive tonic GABAergic and glutamatergic synaptic inputs.

BIC decreased the firing rate in 9 (i.e. 20%) neurons from $1.2 \pm 0.13$ to $0.48 \pm 0.15$ action potentials/s, indicating that a small population of DMV neurons do not receive a direct tonic GABAergic input but, rather, receive inputs from inhibitory non-GABAergic neurons that are themselves subject to a tonic GABAergic influence (Figure 3). In these neurons, perfusion with KYN, in the presence of BIC, further decreased or blocked the firing rate in 4 (i.e. 44%; from $0.22 \pm 0.13$ to $0.12 \pm 0.12$ action potentials/s; figure 3A,C), suggesting that some DMV neurons receive a direct tonic glutamatergic input (Figure 3C).

In the remaining five neurons in which BIC decreased the firing rate, perfusion with KYN increased in 1 (from 0.77 to 0.9 action potentials/s) and had no effect on the firing rate of the 4 remaining (i.e 44%) neurons (Figure 3A).

The remaining 18 (i.e. 29%) of all the neurons tested with BIC) neurons tested did not respond to perfusion with BIC with an alteration in firing rate (Figure 4), suggesting that these neurons are not under tonic GABAergic influence. The firing rate of these neurons was $0.99 \pm 0.04$ and $1.0 \pm 0.04$ action potentials/s before and after application of BIC, respectively. In these 18 neurons, application of KYN decreased the firing rate in 5 (i.e. 28%; from $0.95 \pm 0.05$ to $0.68 \pm 0.18$ action potentials/s; figure 4B), increased it in 6 (i.e. 33%, from $1.05 \pm 0.07$ to $2.0 \pm 0.33$ action potentials/s; figure 4C) and had no effect on the firing rate of 7 (i.e. 39%; not
shown) neurons. These data indicate that the majority of neurons that are not under tonic GABAergic influence are modulated by tonic glutamatergic inputs and that some of these inputs impinge on an inhibitory non GABA-ergic interneuron (Figure 4B,C).

Nineteen of the neurons that were tested with BIC and KYN were identified by fluorescence labeling as gastric-projecting neurons. The percentage of gastric-projecting neurons that had their firing rate modulated in response to perfusion with BIC and KYN was not different from those of the unidentified neurons (Data not shown, but see Table 1; p>0.05).

In summary, these data suggest that the firing rate of the majority of DMV neurons is modulated by tonic GABAergic inputs, and that the firing rate of a large percentage of DMV neurons is also modulated by tonic glutamate inputs.

**Kynurenic acid (KYN) modulates the firing rate of the majority of DMV neurons**

Basal firing rate of neurons that were tested with KYN was $1.04 \pm 0.03$ action potentials/s. Perfusion of the slice with 1mM KYN altered the firing rate in 37 of 58 (64%) tested neurons, indicating that the firing rate of these DMV neurons is modulated by tonic glutamatergic inputs. In the remaining 21 (36%) neurons, perfusion with KYN did not alter their firing rate, indicating that this neuronal subpopulation is not under a tonic glutamatergic influence.

KYN decreased the firing rate in 9 (i.e. 24%; from $1.06 \pm 0.07$ to $0.3 \pm 0.06$ action potentials/s) and abolished it in 16 of the 37 (i.e. 43%; from $1.03 \pm 0.07$ to $0 \pm 0$ action potentials/s) responsive neurons (figure 5A,B), indicating that tonic excitatory glutamate inputs affect, perhaps even control, the firing rate of DMV neurons. In the continuous presence of KYN, application of BIC increased the firing rate in 3 (i.e. 12%; from $0.3 \pm 0.06$ to $0.53 \pm 0.08$ action potentials/s; figure 5C), decreased it in 2 (i.e. 8%; from $0.33 \pm 0.13$ to $0.13 \pm 0.13$ action potentials/s figure 5D) and had no additional effect on 20 neurons (i.e. 80%; not shown). The proposed sites of action of KYN and BIC are depicted in figure 5B-D.

Perfusion with KYN increased the firing rate in 12 (32%) neurons (from $1.09 \pm 0.04$ to $2.03 \pm 0.17$ action potentials/s), suggesting that the firing rate of a subpopulation of DMV neurons is modulated by tonic glutamatergic inputs impinging upon inhibitory synapses (Figure 6). In these neurons, application of BIC further increased the firing rate in 6 (i.e. 50%; from 2.39
± to 4.90 ± 0.1 action potentials/s; figure 6B), decreased it in 2 (i.e. 18%; from 1.77 ± 0.37 to 1.18 ± 0.08 action potentials/s) and did not affect the firing rate in 4 neurons (i.e. 32%). These data indicate that many of these DMV neurons are also under tonic GABAergic influence, whereas, the BIC–induced decrease in firing rate suggests the presence of a non-GABAergic inhibitory neuron controlled by a glutamate synapse. The proposed sites of action of KYN and BIC are depicted in figure 6B,C).

Perfusion with KYN had no effect on the firing rate of 21 (i.e. 36%) of DMV neurons Figure 7A), indicating that these neurons do not receive tonic glutamatergic inputs. The firing rate of these neurons was 0.98 ± 0.03 action potentials/s in control conditions and 0.99 ± 0.05 action potentials/s after application of KYN. In these neurons, further perfusion with BIC increased the firing rate in 6 (i.e. 29%; from 0.93 ± 0.13 to 2.23 ± 0.53 action potentials/s; figure 7B), decreased it in 6 (i.e. 29%; from 1.17 ± 0.18 to 0.72 ± 0.08 action potentials/s; figure 7C) and had no effect on the firing rate of the 9 remaining neurons (i.e. 42%; data not shown). These data suggest that the firing rate of neurons that do not receive tonic glutamatergic input is modulated by tonic GABA inputs impinging on either excitatory or inhibitory synapses. The proposed sites of action of BIC and KYN are depicted in figure 7B,C).

Twenty-two neurons that were tested with KYN and BIC were identified as gastric-projecting neurons. The percentage of gastric-projecting neurons that have their firing frequency modulated in response to KYN or BIC was not different from that of the unidentified neurons (p>0.05).

In summary, these data demonstrated that the firing rate of the majority of DMV neurons is modulated by both tonic GABAergic and glutamatergic inputs. The finding that prior application of KYN decreased the percentage of neurons affected by BIC suggests that tonic GABAergic inputs affect DMV neurons through excitatory, glutamatergic interneurons. Moreover, the finding that KYN increases the firing rate of a subpopulation of DMV neurons but this increased activity was not affected by BIC suggests that this subpopulation receives a tonic glutamatergic input that controls an inhibitory, non-GABAergic neuron, most likely utilizing catecholamines activating an α2 adrenoreceptor (Babic et al, unpublished data).

DISCUSSION
In this study, we have shown that, in a brainstem slice preparation, 1) the firing rate of
most DMV neurons is modulated by tonic GABAergic and glutamatergic inputs; and, 2) there
are several permutations in the arrangements of the GABA- and glutamatergic inputs onto DMV
neurons.

These data support the concept of a brainstem neuronal circuitry that is arranged,
possibly in an organ- or function-specific, as well as a finely tuned manner, which permits a
precise modulation of the vagal motor output to the GI tract.

Our conclusions are based on the observation that, using an approach as simple as the
sequential treatment with the GABA\textsubscript{A} receptor antagonist bicuculline (BIC) and the non-selective
ionotopic glutamate receptor antagonist kynurenic acid (KYN), we uncovered the presence of
several different neuronal circuits in which BIC and KYN have excitatory, inhibitory or no effects
on DMV activity.

The dense synaptic network between the NTS and the DMV uses mainly GABA and
 glutamate, as well as catecholamines, as neurotransmitters (23; 31; 49; 56; 59) and serves as
the final CNS locus for the control of vagal output to the subdiaphragmatic viscera. The
complexity of the adaptive tasks necessary to provide an adequate control of bodily
homeostasis is achieved by integrated levels of modulation that require coordinated actions at
many sites along the brain-gut axis. The diverse arrangements of these synaptic connections
between GABAergic and glutamatergic neurons in NTS-DMV circuits ensure that the majority of
parasympathetic homeostatic functions can be regulated by DMV neurons using only two simple
neurotransmitters. We hypothesize that subtle differences, such as those obtained by variations
in synapses impinging on DMV neurons, e.g. tonic vs phasic, glutamate vs GABA, represent a
metabolically inexpensive means by which vagal output to the GI tract can be precisely tuned to
ever-changing physiological or environmental cues.

Indeed, it is not too far-reaching to assume that autonomic brainstem circuits may have
multiple levels of pathway specificity, from the sensory neurons and terminals, to second order
NTS and motoneurons of the DMV to the effector organ, encompassing all aspects of the vago-
 vagal circuit. Such a concept implies that every autonomic function is controlled by distinct sets
of neurons, each of which is committed to one particular type of response and comprises a
“dedicated” neuronal circuit. This type of neuronal organization may represent specialization or
segregation into specific functional lines and implies a “task matching” capability where subsets
of parasympathetic (and sympathetic) brainstem neurons integrate vital cardiac, respiratory and GI functions. This concept of “task matching” organization is supported by solid evidence, particularly with regard to NTS neurons that receive inputs from baroreceptor sensitive afferent fibers of the aortic depressor nerve (5-7; 26; 39; 43). With respect to specifically GI circuits and mechanisms, this concept is starting to receive attention and is supported by both anatomical and physiological data (reviewed in (20; 57)).

The in vivo observations of Sivarao and colleagues (53), in which the significant increase of gastric motility obtained by microinjection of BIC in the DVC was blocked by microinjection of KYN, was essential in providing evidence of the glutamatergic modulation of a tonic GABAergic input to DMV neurons. Further, Mussa and Verberne reported that, as with vagal control of gastric tone, pancreatic exocrine secretion is also held in check by GABAergic tonic inputs onto brainstem vagal motoneurons (44). When transposed to the brainstem slice preparation, these in vivo observations predict that: i) perfusion of the slice with BIC increases the firing rate of DMV neurons; ii) KYN perfusion has no effect on the firing rate of DMV neurons; and, iii) BIC would have been ineffective when perfused in the presence of KYN.

Our results suggest that these hypothetical scenarios can, indeed, be reproduced in the brainstem slice preparation. Our results also suggest, however, that the aforementioned scenarios should not be taken as the only potential circuitry arrangements, as we have uncovered the presence of synaptic arrangements that would not explain the in vivo results of Sivarao (53) and Mussa (44). In particular, we should point out that the data obtained from labelled gastric-projecting and those obtained from unlabelled, but most likely gastric-projecting, DMV neurons have revealed a similar synaptic arrangement between the two groups. This observation would then argue in favor of the generalized use of the brainstem slice preparation as a model to study the synaptic arrangement of the GI vagal circuits.

Although several differences between the in vivo and in vitro experimental approaches have to be taken into consideration, the most likely explanation of the several permutations in the slice preparation is that DMV neurons with different functions, for example those devoted to the modulation of gastric motility versus those devoted to the control of gastric acid secretion, respond differently to BIC and KYN because distinct circuits serve diverse functions. As yet, neuronal tracing techniques cannot distinguish between different physiological functions, such as, for example, gastric motility versus secretion or pancreatic endocrine versus exocrine secretion, therefore most likely the labeled DMV neurons represent a functionally mixed neuronal population.
It has also to be taken into consideration that in vivo studies report the physiological outcome of effects upon several neuronal populations, which may include inhibitory, excitatory and unaffected single neurons. Conversely, the present study measured the firing rate of single DMV neurons identified as per their target organ, but not their function. The correlation between neuronal responses to BIC and KYN with their GI function has still to be investigated.

In the present study, we demonstrated that tonic GABA and glutamate inputs have different effects on subpopulations of DMV neurons, suggesting that these neurons contain distinct complements of GABA_\text{A} and glutamate receptors, as suggested previously (12; 31; 40; 41). Note that we examined only ionotropic receptors; the presence of GABA and glutamate metabotropic receptors in vagal brainstem circuits (17; 21; 35; 38; 47; 48; 60) adds another potentially larger level of complexity. Indeed, in the present study we did not examine the contribution of metabotropic receptors to the basal firing rate of DMV neurons and thus we cannot exclude their activation; however, we did not observe any significant effect on the DMV membrane induced by the GABA_\text{B} antagonist, saclofen (17), or the metabotropic group II and III antagonists, EGLU and AP-4 (21). Evidence from several groups implicates catecholamines, particularly norepinephrine (NE), as an important neurotransmitter between NTS and DMV (10; 36; 42; 49). Acting via \(\alpha_2\) adrenoreceptors, NE may well be the non-GABAergic inhibitory neurotransmitter proposed in the present study. In fact, perfusion with the \(\alpha_2\) adrenoceptor antagonist, yohimbine, decreased partially the firing rate in a subset of DMV neurons pretreated with KYN (Babic et al., unpublished). These \(\alpha_2\) adrenoceptor-mediated effects, however, were not observed in DMV neurons perfused with yohimbine only (42), indicating that catecholaminergic neurotransmission onto the DMV may be driven through other, yet undefined, synapses.

The diverse arrangements of these GABA and glutamate ionotropic circuits may also contribute to modulate sensitivity of DMV neurons to other neurotransmitters. Indeed, several neurotransmitters preferentially affect either excitatory or inhibitory currents in the DMV. Met-enkephalin and pancreatic polypeptides, for example, alter the glutamatergic currents onto DMV neurons, but have no effect on GABAergic inputs (14; 19), unless the appropriate conditions ensue (15; 22). Similarly, perfusion of DMV neurons with cannabinoids appear to produce a depolarization-induced suppression of inhibitory GABA- but not excitatory glutamatergic currents (51). The presence or absence of GABA_\text{A} and/or glutamate receptors on a particular neuron could, therefore, dictate whether that neuron would respond to other neurotransmitters. Since both GABA and glutamate inputs converge and modulate the activity of single DMV
neurons, this implies that the activity of a given brainstem circuit is also differentially regulated by the discrete distribution of the several neurotransmitters’ receptors.

The convergence of tonic or phasic GABA and glutamate inputs onto DMV neurons when combined with the different expression of receptor subtypes may, therefore, provide a metabolically inexpensive basis for the integration of inputs from multiple areas of the CNS or directly from the visceral organs onto single DMV neurons.

Although there is undisputable evidence of glutamatergic synapses impinging on GABAergic neurons in NTS (5; 32), these glutamatergic inputs from the tractus solitarius onto the NTS neurons are unlikely to be those responsible for the observed effects of KYN. In fact, contrary to cardiovascular afferent fibers comprising the baroreflex, gastrointestinal vagal fibers impinging onto NTS neurons are rarely observed to have a baseline tonic activity (11) sufficient to drive the activity of NTS-DMV connections and fully activate vago-vagal reflexes.

In summary, we have demonstrated that, in a brainstem slice preparation, tonic excitatory (glutamate) and inhibitory (GABA) inputs modulate the firing rate of the majority of DMV neurons via actions at ionotropic receptors. Furthermore, different combinations of excitatory and inhibitory synaptic inputs results in dramatically different effects of glutamate and GABA on DMV neuronal excitability.

The present data support the hypothesis that brainstem circuitry may be organized, possibly in an organ-specific or function-specific manner, in such a way as to permit precise modulation of vagal motor output to discrete visceral areas. Such a neuronal arrangement strongly support the application of the concept of “task matching” organization to the functional brainstem circuits that control GI functions.
Table 1: Effect of bicuculline and kynurenic acid on the firing rate of DMV neurons. Bicuculline was applied first, followed by kynurenic acid. The numbers represent the number of neurons that exhibited given responses to bicuculline (columns) and kynurenic acid (rows).

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<th>All neurons</th>
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<td></td>
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Table 2: Effect of kynurenic acid and bicuculline on the firing rate of DMV neurons. Kynurenic acid was applied first, followed by bicuculline. The numbers represent the number of neurons that exhibited given responses to kynurenic acid (columns) and bicuculline (rows).

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<tr>
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<tr>
<td>Bicuculline</td>
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FIGURE LEGENDS

Figure 1: Schematic diagram showing glutamate (black), GABA (gray) and inhibitory non-GABAergic (white) neurons impinging on the DMV. Note that we do not mean to imply that the synaptic contacts occur specifically on the dendrites, axons or synaptic terminals, but are depicted in this manner for schematic clarity.

Figure 2: Pie charts (A), representative traces (B-D left) and the proposed neuronal circuits (B-D right) in neurons in which perfusion with bicuculline (BIC) increased the firing rate of DMV neurons.

453A) Perfusion with BIC increased the firing frequency of DMV neurons (N=35) and perfusion with KYN decreased the firing frequency of the majority of these neurons. Note that the pie chart on the right represents subgroups of neurons in which BIC increased the firing frequency.

457B) The increase in firing rate induced by BIC is likely due to antagonism of GABA_A receptors located either on the membrane of the DMV neuron or on tonically active glutamatergic neurons which impinge upon the DMV neuron.

460C) Perfusion with KYN in the presence of BIC decreased the firing rate of DMV neurons (N=19). This response was likely due to KYN-mediated inhibition of a tonic glutamatergic input onto DMV neurons.

463D) Perfusion with KYN in the presence of BIC further increased the firing rate of DMV neurons (N=10), suggesting the presence of tonic glutamatergic synapses impinging onto non-GABAergic neurons.

In all figures, the proposed site of action of bicuculline is indicated by a solid line red square and the proposed site of action of kynurenic acid is indicated by a dotted line yellow square.

Figure 3: Pie charts (A), representative traces (B,C left) and the proposed neuronal circuits (B,C right) in neurons in which perfusion with BIC decreased the firing rate of DMV neurons.

475A) Perfusion with BIC decreased the firing frequency of DMV neurons (N=9), and KYN further decreased the firing frequency in majority of these neurons. The pie chart on the right represents subgroups of neurons in which BIC decreased the firing frequency.

478B) The decrease in firing rate induced by BIC is likely due to antagonism of GABA_A receptors located on the membrane of non-GABAergic inhibitory neurons.

480C) Perfusion with kynurenic acid (KYN) in the presence of BIC further decreased the firing rate of DMV neurons (N=4). This response was likely due to KYN-mediated inhibition of a tonic glutamatergic input onto DMV neurons.

Figure 4: Pie charts (A), representative traces (B,C left) and the proposed neuronal circuits (B,C right) in neurons in which perfusion with BIC did not affect the firing rate (N=18) of DMV neurons.

487A) Perfusion with kynurenic acid (KYN) in the presence of BIC decreased the firing rate of DMV neurons (N=5).

489B) The decrease in the firing rate in response to KYN was likely due to KYN-mediated inhibition of a tonic glutamatergic input onto DMV neurons.
Perfusion with KYN in the presence of BIC increased the firing rate of DMV neurons (N=6), suggesting the presence of tonic glutamatergic synapses impinging onto non-GABAergic neurons.

Figure 5: Pie charts (A), representative traces (B-D left) and the proposed neuronal circuits (B-D right) in neurons in which perfusion with KYN decreased the firing rate of DMV neurons.

Perfusion with KYN decreased (or abolished) the firing frequency of DMV neurons (N=25), and BIC had no additional effect on the firing frequency in majority of these neurons.

The decrease in firing rate induced by KYN is likely due to antagonism of glutamatergic receptors located on the membrane of the DMV neuron.

Perfusion with BIC in the presence of KYN increased the firing rate of DMV neurons (N=3). This response was likely due to BIC-mediated inhibition of a tonic GABAergic input onto DMV neurons.

Perfusion with BIC in the presence of KYN further decreased the firing rate of DMV neurons (N=2), suggesting the presence of tonic GABAergic synapses impinging onto non-GABAergic neurons.

Figure 6: Pie charts (A), representative traces (B,C left) and the proposed neuronal circuits (B,C right) in neurons in which perfusion with KYN increased the firing rate of DMV neurons.

Perfusion with KYN increased the firing frequency of DMV neurons (N=12) and perfusion with BIC further altered the firing frequency of 8 of these neurons.

The increase in firing rate induced by KYN is likely determined by antagonism of tonically active glutamatergic receptors located on the membrane of either GABAergic or inhibitory non-GABAergic neurons.

Perfusion with BIC in the presence of KYN further increased the firing rate of DMV neurons (N=6). This response was likely due to a BIC-mediated inhibition of a tonic GABAergic input onto DMV neurons.

Figure 7: Pie charts (A), representative traces (B,C left) and the proposed neuronal circuits (B,C right) in neurons in which perfusion with KYN did not affect the firing rate (N=21) of DMV neurons.

Perfusion with BIC in the presence of KYN either increased (N=6) or decreased (N=6) the firing rate of DMV neurons (N=6).

The increase in the firing frequency was likely due to BIC-mediated inhibition of a tonic GABAergic input onto DMV neurons.

Perfusion with BIC in the presence of KYN decreased the firing rate of DMV neurons (N=6), suggesting the presence of tonic GABAergic synapses impinging onto non-GABAergic neurons.


18.


19.


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24.


25.


61029. **Fox EA and Powley TL.** Longitudinal columnar organization within the dorsal motor nucleus represents separate branches of the abdominal vagus. *Brain Res* 341: 269-282, 6121985.


A

BIC Decrease 13%

BIC Increase 57%

BIC No Effect 30%

B

Control + BIC Wash Out

20mV

15s

C

Control + BIC + KYN

D

Control + BIC + KYN

Babic et al. Figure 2

BIC Increase

57%

(B)

KYN Increase

29%

(D)

KYN No Effect

18%

(not shown)
A

B

Control + BIC Wash Out

BIC No Effect 30%
BIC Decrease 13% (B)
BIC Increase 57%

KYN Increase 12% (not shown)
KYN Decrease 50% (C)
KYN No Effect 38% (not shown)

B

Control + BIC + KYN Wash Out

Control + BIC + KYN Wash Out

BIC Increase 57%
BIC Decrease 13%
KYN Increase 12%
KYN No Effect 38%
KYN Decrease 50%

BIC Decrease 13%
KYN No Effect 38%
KYN Decrease 50%

B

NTS

GABA (-)
DMV

Glutamate (+)
Inhibitory non-GABA nt (-)

C

NTS

GABA (-)
DMV

Glutamate (+)
Inhibitory non-GABA nt (-)
Babic et al. Figure 4

A

GABA (-) Glutamate (+) Inhibitory non-GABA nt (-)

NTS DMV

15s

B

Control + BIC + KYN

BIC Increase 57%
BIC No Effect 30% (B)
BIC Decrease 13%

KYN Increase 33% (C)
KYN Decrease 28% (B)
KYN No Effect 39% (not shown)

20mV 15s

C

Control + BIC + KYN

BIC Increase 57%
BIC No Effect 30% (B)
BIC Decrease 13%

KYN Increase 33% (C)
KYN Decrease 28% (B)
KYN No Effect 39% (not shown)
Babic et al. Figure 5

A

KYN No effect 36%
KYN Increase 21%
KYN Decrease 43%
BIC Increase 12% (C)
BIC No Effect 80% (not shown)

B

Control + KYN Wash Out 20mV

C

Control + KYN + BIC

D

Control + KYN + BIC

KYN Increase 21%
KYN No effect 36%
KYN Decrease 43%
BIC Increase 12%
BIC No Effect 80%
BIC Decrease 8%

(B) (C) (D) (not shown)
A

- KYN Decrease 43%
- KYN Increase 21%
- KYN No effect 36%
- BIC Decrease 17% (not shown)
- BIC Increase 50% (C)
- BIC No Effect 33% (not shown)

B

Control + KYN Wash Out 20mV 15s

C

Control + KYN + BIC

Babic et al. Figure 6

NTS DMV

KYN Increase 21% (B)

KYN No effect 36%

BIC Decrease 17% (not shown)

BIC Increase 50% (C)

BIC No Effect 33% (not shown)

Control + KYN + BIC

NTS DMV

GABA (-) Glutamate (+) Inhibitory non-GABA nt (-)

BIC Increase 50%

BIC No Effect 33%

BIC Decrease 17%

KYN Increase 21%

KYN No effect 36%
Babic et al. Figure 7

A

- KYN Increase 21%
- KYN Decrease 43%
- KYN No effect 36%

B

15s

Control + KYN + BIC 20mV

KYN Increase 21%
KYN No effect 36%
KYN Decrease 43%

BIC Decrease 29%
BIC Increase 28%
BIC No Effect 43%

B

Control + KYN + BIC

BIC Increase 28%
BIC Decrease 29%
BIC No Effect 43%

C

Control + KYN + BIC