Influence of Vagotomy on Monosynaptic Transmission at Second-Order Nucleus Tractus Solitarius Synapses

Jessica B. Swartz1 and Daniel Weinreich1,2
1Program in Neuroscience and 2Department of Pharmacology and Experimental Therapeutics, University of Maryland, School of Medicine, Baltimore, Maryland

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INTRODUCTION

The nucleus tractus solitarius (NTS), located in the brain stem, is critical to the receipt, integration, and transmission of primary sensory signals coming from visceral organs (Loewy 1990). These sensory signals, coded in the pattern of action potential activity, are sent to the NTS via axons of the vagus nerve where they are subsequently translated and processed by NTS synapses. Thus second-order NTS neurons do not merely relay vagal activity to higher brain centers. Instead they incorporate afferent information with activity in local circuits and upstream efferent signals to control autonomic homeostasis, the maintenance of equilibrium between the sympathetic and parasympathetic systems (Andresen and Kunze 1994; Bonham et al. 2006). Alterations to the information coming from vagal afferents could change synaptic communication to NTS neurons, modifying upstream circuits and throwing off autonomic homeostatic balance.

There are many serious disorders, such as asthma and hypertension, that can alter the excitability of peripheral nerve terminals of the vagus nerve. Several studies have revealed that analogous pathologies produce physiological changes at the level of the NTS. For example, in response to chronic intermittent hypoxia, NTS neurons adjust their electrophysiological properties to compensate for elevated afferent impulse activity (Kline et al. 2007). Similarly, NTS neurons from ozone-exposed primates not only have a more depolarized membrane potential, but electrical stimulation of primary afferents in the tractus solitarius (ST) elicits smaller excitatory postsynaptic responses when compared with controls (Chen et al. 2001). Each of these studies revealed that NTS neurons and their primary afferent synapses can scale their electrophysiological properties to compensate for increased or decreased activity, a form of homeostatic plasticity (Bonham et al. 2006; Turrigiano and Nelson 2000).

In addition to pathophysiological disorders that cause insult to the peripheral sensory nerve terminals of the vagus, there are instances when the vagus nerve itself becomes injured. Heart and lung transplant, as well as treatments for gastrointestinal disorders, result in either full or partial section of the vagus nerve (vagotomy). Although vagotomy is increasingly being used as a treatment for many disorders, little is known about side effects and long-term changes to CNS processing following the procedure. There is, however, evidence that vagotomy alters glucose metabolism in the gut, and this modification is mediated through NTS circuits (Wang et al. 2008). Furthermore, vagotomy can produce profound changes in the electrophysiological properties of the cell bodies of the vagus nerve (nodose ganglion neurons). These changes include hypoxic-ability (Lancaster et al. 2000) associated with a reorganization of sodium currents (Lancaster and Weinreich 2001) and the appearance of Ca2+-activated Cl− currents (Lancaster et al. 2002). Although these findings make a good case for the possibility of synaptic plasticity in the central portion of the vagal circuit, changes in NTS synaptic communication due to vagotomy have not been examined.

We unilaterally vagotomized Sprague-Dawley rats distal to the nodose ganglion to investigate changes in synaptic transmission in the NTS. We hypothesized that severing the vagus nerve, and thus incoming vagal action potential activity to monosynaptic NTS neurons, would cause a homeostatic, compensatory increase in synaptic efficacy at this first synapse. Contrary to our expectations, we discovered that a population of NTS neurons preferentially demonstrated synaptic depres-
sion in response to vagotomy. Furthermore, vagotomy caused an increase in the percentage of these neurons. Our results suggest that vagotomy, rather than simply blocking action potential activity in vagal afferents, may be eliciting a complicated, injury-induced response at NTS synapses.

METHODS

Ethical approval

All animal procedures were approved by the Institutional Animal Care and Use Committee of University of the Maryland, Baltimore, MD.

Vagotomy

All experiments were performed on male or female Sprague-Dawely rats (90–350 g, Harlan). Sixteen rats were anesthetized with inhaled isoflurane anesthesia (2–3.5%). An incision was made down the midline of the throat, and the sternohyoideus and omohyoideus muscles were careful retracted to expose the left vagus nerve. After carefully detaching the vagus nerve from the carotid artery, the sympathetic nerve trunk and surrounding connective tissue, an 1–2 cm section of the left vagus nerve was removed. The wound was then closed and sutured shut. Animals were allowed to recover for 6–30 days, after which they were killed (see Slice preparation), and the persistence of a cut vagus nerve was visually confirmed. Because there were no statistical differences within the 6- to 30-day range for any tested parameters, all vagotomized data were pooled. Three sham animals underwent the same operation without severing the vagus nerve. Parameters from sham animals were not statistically different from those of control, unoperated animals (21 rats); therefore sham and control data were combined.

Slice preparation

Under deep inhaled isoflurane anesthesia (4%), animals were killed by decapitation, and the brain stem was removed. A single 250-μm-thick horizontal brain stem slice (Doyle et al. 2004) containing the NTS was cut in ice-cold oxygenated artificial cerebrospinal fluid (ACSF) using a vibrating microtome (Leica, Wetzlar, Germany) with a sapphire knife. The ACSF was composed of (in mM) 125 NaCl, 25 NaHCO3, 1.2 KH2PO4, 3 KCl, 1.2 MgSO4, 2 CaCl2, and 10 glucose oxygenated with 95% O2-5% CO2, pH 7.4. Immediately after cutting, the slice was transferred to the recording chamber and perfused with continuously oxygenated 30–32°C ACSF and allowed to recover for ≥20 min.

Whole cell recording

Patch and stimulating electrode placement was visualized using near-infrared differential interference contrast microscopy under both ×4 and ×40 objectives attached to a fixed-stage, upright microscope (Nikon Eclipse, E600 FN, Melville, NY). Whole cell voltage- and current-clamp recordings were acquired from monosynaptic NTS neurons medial to the solitary tract (ST; Fig. 1) using an Axopatch 200A amplifier (Axon Instruments, Union City, CA). All records were filtered at 5 kHz and digitized at 20 kHz using a DigiData 1322A interface (Axon Instruments). Data were acquired with pClamp 9 (Axon Instruments) software. Recording patch electrodes (resistances of 3–8 MΩ) were filled with a solution composed of (in mM) 2 NaCl, 2 MgCl2, 10 EGTA, 10 HEPES, 0.2 NaGTP, 3 MgATP, 1 CaCl2, and 144 K-gluconate, pH 7.2. We recorded from neurons located medial to the solitary tract, caudal to the dorsal vagal motor nucleus, and rostrolateral to the area postrema. Only neurons with input resistances >200 MΩ and holding currents <100 pA were accepted for study. Series resistance was not compensated. Membrane potential values were corrected for the liquid junction potential (~14 mV). Bicuculline methiodide (10 μM) was present in the ACSF of all experiments to remove the influence of inhibitory GABA_A communication.

Solitary tract stimulation

To elicit synaptic responses, a homemade bipolar stimulating electrode (150 μm tip width) was placed on the afferent axons of the solitary tract (ST) ~1–3 mm from the recorded NTS neurons...
(Fig. 1). Originally, to stimulate the ST, constant voltage (10–100 V, 0.15 ms) was delivered through a stimulus isolation unit driven by a Grass S88 stimulator (Astro-Med, West Warwick, RI). After a few months, however, we switched to a constant current stimulus isolation unit (Astro-Med). There were no statistically significant differences between responses elicited by the constant voltage or constant current sources. Stimulating current amplitudes ranged from 0.01 to 1 mA for a duration of 0.15 ms. Current intensity was gradually increased until an excitatory postsynaptic current was evoked (evEPSC) and stimulation intensity was set 10% above threshold. Activation of monosynaptic synapses elicited an all-or-none EPSC. We determined whether synaptic responses were monosynaptic by measuring the average SD of the synaptic latency (jitter) of 10 evEPSCs in response to ST stimulation. Cells with jitter values of $<200 \mu s$ were considered monosynaptic (Doyle et al. 2004) (see Fig. 2). Bursts of five stimuli were delivered at 50 Hz at 5-s intervals. Only monosynaptic evEPSCs were accepted for analysis.

Analysis of passive and active membrane properties

Active membrane properties were recorded in current-clamp mode. Rheobase (the current threshold to elicit an action potential) was determined by applying incremental 50-pA depolarizing current pulses until an action potential occurred. Neuronal excitability was evaluated by applying a 750-ms depolarizing current step at rheobase and counting the number of action potentials that occurred during the pulse. The passive properties of membrane resistance ($R_m$) and capacitance ($C_m$) were recorded in voltage-clamp mode using the membrane test protocol of pClamp and monitored throughout the experiment.

Drugs

Tetrodotoxin (TTX) was purchased from Calbiochem (Darmstadt, Germany). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Data analysis

Data were analyzed using SigmaPlot (Systat Software, San Jose, CA) and MiniAnalysis (Synaptosoft, Decatur, GA) software. The minimum amplitude threshold for detecting spontaneous and mini events was set at 10 pA, as baseline noise was generally between 5 and 8 pA. Because most data did not pass tests for normality, statistical comparisons were made using the Mann-Whitney rank sum test and data are presented as medians followed by the 25–75% confidence interval unless otherwise noted. When data were normally distributed, comparisons were made using the Student’s t-test, and values were presented as means ± SE. A $P$ value of $<0.05$ indicated statistical significance.

RESULTS

Vagotomy causes a decrease in synaptic efficacy at monosynaptic NTS synapses

Homeostatic theory predicts that postsynaptic neurons scale their electrophysiological properties to compensate for long-term increases or decreases in presynaptic activity (Turrigiano and Nelson 2000). Indeed vagal NTS synapses and autonomic reflexes have been shown to undergo homeostatic plasticity in response to a number of different presynaptic insults (see review by Bonham et al. 2006). In accordance with homeostatic compensation, we therefore expected an electrical stimulus to the ST to elicit an increase in the synaptic efficacy of monosynaptic NTS neurons following vagotomy. This increase synaptic efficacy would be characterized by an augmented evEPSC.

Contrary to our expectations, 6–30 days after vagotomy ($n = 43$), we observed a decrease in synaptic efficacy when compared with controls ($n = 66$). The reduction in synaptic efficacy was revealed by a smaller median amplitude of the evEPSC after vagotomy [Fig. 3, A and B; $P < 0.05$; $–157$ (–94 to –253) pA vs. $–121$ (–59 to –188) pA; control vs. vagotomy, respectively]. To investigate whether this change was pre- or postsynaptic in nature, we compared the passive and active membrane properties of control and vagotomized NTS neurons as well as the spontaneous excitatory synaptic potentials of these two groups (see Table 1 for values). There were no significant differences between control and vagotomized groups when comparing average membrane resistance, capacitance, or potential. Similarly, there was not a significant difference in the excitability of postsynaptic NTS neurons, measured by counting the number of action potentials elicited by rheobase current. In a similar vein, both mean synaptic latency and median jitter were not statistically significant when comparing control versus vagotomized synapses. There was also no significant difference between the decay times of the first evEPSC of control and vagotomized synapses. Furthermore, there were no significant differences between the median spontaneous frequency and amplitude. In a subgroup of neurons, we also measured miniature EPSCs (mEPSCs) in the presence of TTX (2 μM). The frequency and amplitude of mEPSCs from the vagotomized group ($n = 4$) were not significantly different when compared with controls ($n = 8$). The absence of any electrophysiological changes to the postsynaptic neurons, along with the lack of differences between mEPSC amplitudes following vagotomy, suggests that the decrease in synaptic efficacy after vagotomy is due to presynaptic rather than postsynaptic alterations.

To ascertain whether the observed decrease in synaptic efficacy following vagotomy could be explained by a less efficient stimulus delivery to the ST, we performed stimulus
intensity measurements in a subgroup of neurons. There was no significant difference between the mean stimulus threshold required to elicit an evEPSC in control (n = 24) and vagotomized (n = 15) neurons [P > 0.05; 0.7 ± 0.26 vs. 0.54 ± 0.15 (SE) mA; Student’s t-test].

Two distinct populations of monosynaptic NTS responses

Monosynaptic NTS synapses are known to be glutamatergic and have a high probability of release (Bailey et al. 2006). In response to a train of five electrical stimuli (50 Hz) presented to the presynaptic axons of the ST, the amplitudes of evEPSCs of monosynaptic synapses display a characteristic frequency-dependent depression. Although the first evEPSC responds in an all-or-none manner with high fidelity, failing to respond to stimulation <1% of the time (see Fig. 4) (cf. Bailey et al. 2008), the subsequent four evEPSCs not only progressively decrease in amplitude but have a higher probability of failure. The degree of synaptic failure exhibited during the 50-Hz train of stimuli has been used to define two separate classes of monosynaptic EPSCs evoked by electrical ST stimulation (Andresen and Peters 2008). In accordance with this previous study, we designated synapses with less than a 5% failure rate as “low-failure synapses” and those with a failure rate equal to or over 5% as “high-failure synapses.” These percentages are based on the total number of failures of 50 possible responses (5 stimuli over 10 trials). The 5% cut-off point was chosen as a conservative middle point between a restrictive zero failure rate and the average percentage of responses of control synapses that fail in response to the fifth stimulation (12%; see Fig. 4).

TABLE 1. Effects of vagotomy on passive, active, and synaptic properties

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vagotomy</th>
</tr>
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<tbody>
<tr>
<td>P</td>
<td></td>
<td>354 (268–490)*</td>
</tr>
<tr>
<td>Cm, MΩ</td>
<td>0.9 66</td>
<td>21 (18–25)*</td>
</tr>
<tr>
<td>Vm, mV</td>
<td>0.4 66</td>
<td>−64 (−69 to −59)*</td>
</tr>
<tr>
<td>Excitability, No. of spikes</td>
<td>0.9 66</td>
<td>33 ± 2§</td>
</tr>
<tr>
<td>Latency, ms</td>
<td>0.8 66</td>
<td>3.9 ± 0.2§</td>
</tr>
<tr>
<td>Jitter, μs</td>
<td>0.6 66</td>
<td>83 (63–116)*</td>
</tr>
<tr>
<td>spEPSC frequency, Hz</td>
<td>1.0 66</td>
<td>4.7 (3 to 9)*</td>
</tr>
<tr>
<td>spEPSC amplitude, pA</td>
<td>0.9 66</td>
<td>−28 (−22 to −40)*</td>
</tr>
<tr>
<td>mEPSC frequency, Hz</td>
<td>0.7 8</td>
<td>8 ± 2§</td>
</tr>
<tr>
<td>mEPSC amplitude, pA</td>
<td>0.8 8</td>
<td>−34 ± 4§</td>
</tr>
<tr>
<td>evEPSC decay time, ms</td>
<td>0.5 12</td>
<td>3.1 ± 0.2§</td>
</tr>
</tbody>
</table>

Passive, active and synaptic properties in the nucleus tractus solitarius are unchanged following vagotomy. $R_m$, membrane resistance; $C_m$, membrane capacitance; $V_m$, membrane potential; spEPSC, spontaneous excitatory postsynaptic current (EPSC); mEPSC, miniature EPSC recorded in the presence of TTX. *, median (25–75% confidence interval). §, mean ± SE. n, sample size.
In control animals, 70% of the monosynaptic synapses were in the low-failure group \( (n/46/66) \), leaving 30% of synapses in the high-failure group \( (n/20/66) \).

**Low- and high-failure synapses show differences in synaptic efficacy**

In response to ST stimulation, high-failure synapses exhibited a significantly smaller initial evEPSC than those recorded from low-failure synapses \( [F. 4, A and B; P < 0.05; \sim -88 (-125 to -297) pA vs. -184 (-66 to -150) pA] \). The latency of the initial evEPSC response is also significantly different between low- and high-failure synapses. High-failure synapses had a significantly longer mean latency when compared with low-failure responses \( [F. 4 C; P < 0.05; 3.66 \pm 0.2 \text{ vs. } 4.59 \pm 0.3 \text{ (SE) ms}; \text{Student’s} \ t\text{-test}; \text{low vs. high failure, respectively}] \).

Otherwise, these two populations of NTS neurons had no significant differences in their median jitter (see Table 2 for values), membrane resistance, membrane capacitance, or membrane potential. Furthermore, there were no significant differences in average spontaneous EPSC frequencies or amplitudes. In a subpopulation of neurons, we compared the stimulus intensity required to elicit an evEPSC between low \( (n = 17) \) and high \( (n = 7) \) failure synapses. There was no significant differences in average spontaneous EPSC frequencies or amplitudes. In a subpopulation of neurons, we compared the stimulus intensity required to elicit an evEPSC between low \( (n = 17) \) and high \( (n = 7) \) failure synapses. There was no significant differences in average spontaneous EPSC frequencies or amplitudes. In a subpopulation of neurons, we compared the stimulus intensity required to elicit an evEPSC between low \( (n = 17) \) and high \( (n = 7) \) failure synapses. There was no significant differences in average spontaneous EPSC frequencies or amplitudes. In a subpopulation of neurons, we compared the stimulus intensity required to elicit an evEPSC between low \( (n = 17) \) and high \( (n = 7) \) failure synapses. There was no significant differences in average spontaneous EPSC frequencies or amplitudes. In a subpopulation of neurons, we compared the stimulus intensity required to elicit an evEPSC between low \( (n = 17) \) and high \( (n = 7) \) failure synapses. There was no significant differences in average spontaneous EPSC frequencies or amplitudes. In a subpopulation of neurons, we compared the stimulus intensity required to elicit an evEPSC between low \( (n = 17) \) and high \( (n = 7) \) failure synapses. There was no significant differences in average spontaneous EPSC frequencies or amplitudes. In a subpopulation of neurons, we compared the stimulus intensity required to elicit an evEPSC between low \( (n = 17) \) and high \( (n = 7) \) failure synapses. There was no significant differences in average spontaneous EPSC frequencies or amplitudes. In a subpopulation of neurons, we compared the stimulus intensity required to elicit an evEPSC between low \( (n = 17) \) and high \( (n = 7) \) failure synapses. There was no significant differences in average spontaneous EPSC frequencies or amplitudes. In a subpopulation of neurons, we compared the stimulus intensity required to elicit an evEPSC between low \( (n = 17) \) and high \( (n = 7) \) failure synapses. There was no significant differences in average spontaneous EPSC frequencies or amplitudes. In a subpopulation of neurons, we compared the stimulus intensity required to elicit an evEPSC between low \( (n = 17) \) and high \( (n = 7) \) failure synapses. There was no significant differences in average spontaneous EPSC frequencies or amplitudes. In a subpopulation of neurons, we compared the stimulus intensity required to elicit an evEPSC between low \( (n = 17) \) and high \( (n = 7) \) failure synapses. There was no significant difference...
difference in median stimulus threshold between the two groups \( [P > 0.05; 0.08 (0.05–0.4) \text{ vs. } 0.06 (0.02–0.7) \text{ mA}] \).

**Vagotomy causes an increase in both overall synaptic failures and high-failure synapses**

Low- and high-failure synapse populations were also detected in vagotomized animals (Fig. 5A). As was observed with control neurons, the median initial evEPSC amplitude from the high-failure vagotomy group \((n = 24)\) was significantly smaller than from the low-failure group [Fig. 5B; \( n = 19; P < 0.05; -61 (-42 \text{ to } -110) \text{ vs. } -188 (-136 \text{ to } -281) \text{ pA} \)]. Also similar to the control group, the mean latency for vagotomized low-failure synapses was significantly smaller than for vagotomized high-failure synapses (Fig. 5C; \( P < 0.05; 3.4 \pm 0.3 \text{ vs. } 4.5 \pm 0.3 \text{ ms}; \text{ Student's } t\)-test).

As segregating the synapses into low- and high-failure groups was somewhat arbitrary, we also examined the overall percentage of synaptic failures after each stimulus shock in control and vagotomy without distinguishing between failure types. Without discriminating between failure groups, vagotomy profoundly affected the overall percentage of failures following the 50-Hz train of ST stimuli. Synapses from vagotomized animals had a significantly increased overall failure rate in response to all stimulus shocks excluding the first (Fig. 6A; see Table 3 for values).

Because the percentage of synaptic failures increased, we examined whether there was a shift in the population to more high-failure synapses following vagotomoy. Indeed, the percentage of vagotomized high-failure synapses nearly doubled to 56% \((n = 24/43)\) when compared with controls \((30%; n = 20/66; \text{ Fig. 6B})\). Interestingly, when we counted the failures after each stimulus and compared the overall failure rate of control high-failure and vagotomy high-failure synapses, we discovered that vagotomy high-failure synapses have more failures when compared with control high-failure synapses (Fig. 6C; see Table 4 for values). These combined results suggest that vagotomy causes a decrease in synaptic efficacy by both increasing the overall percentage of synaptic failures and shifting the population of NTS synapses toward those that fail more frequently.

To test whether the decrease in synaptic efficacy following vagotomy could be explained by an increase in the percentage of high-failure responses, we compared low- and high-failure synapses across treatment groups using the nonparametric
Kruskal-Wallis ANOVA (Fig. 7). Because the difference among the groups was significant ($P < 0.05$), we used Dunn’s pairwise multiple comparison procedure to tease out the effects of vagotomy. There was no significant difference between the median initial evEPSC for low-failure groups [$P > 0.05$; $–184 (–125 to –297)$ vs. $–188 (–136 to –281)$ pA; control, $n = 46$, vs. vagotomy, $n = 19$]. Although the median initial evEPSC from the high-failure vagotomy group ($n = 24$) was significantly smaller than that from the low-failure vagotomy group, there was no significant differences between the median first evEPSC of the control high-failure group ($n = 20$) and the vagotomy high-failure group [$P > 0.05$; $–88 (–66 to –150)$ vs. $–61 (–42 to –110)$ pA]. It therefore seems that the significant reduction in the median first evEPSC amplitude between all control and all vagotomized neurons was due to the greater number of high-failure synapses in vagotomized rats. This increase in high-failure synapses following vagotomy shifted the overall median initial evEPSC amplitudes to smaller values. It is noteworthy, however, that although it was not statistically significant, the median first evEPSC from high-failure, vagotomized synapses trended toward smaller values than the median evEPSC recorded from high-failure synapses in control neurons (Fig. 7).

These results provide initial insights into the effects of vagotomy on monosynaptic transmission in the NTS. Vagotomy decreases the efficacy of NTS synapses by increasing the percentage of high-failure synapses at the expense of low-failure synapses. While the mechanisms of this change remain unknown, the results from our experiments examining passive and active properties of the postsynaptic neurons as well as the

**TABLE 3. Percentage of total failures is increased following vagotomy**

<table>
<thead>
<tr>
<th>Stimulus Shock</th>
<th>Percent Total Failures of Control Synapses</th>
<th>Percent Total Failures of Vagotomized Synapses</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 ± 0.6</td>
<td>2 ± 0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>3 ± 0.9</td>
<td>10 ± 2.5</td>
<td>0.004</td>
</tr>
<tr>
<td>3</td>
<td>7 ± 1.7</td>
<td>22 ± 4.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>10 ± 2.0</td>
<td>25 ± 4.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5</td>
<td>12 ± 2.0</td>
<td>29 ± 4.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Following vagotomy the percentage of total failures is significantly increased for the second through fifth stimulus shocks during a 50-Hz stimulation train. Percent failures were derived after each stimulus by counting the total number of failures and dividing that number by 10 (for the 10 trials each cell underwent) multiplied by the total number of control ($n = 66$) or vagotomized ($n = 43$) neurons. Values are presented as means ± SE.

**TABLE 4. Percentage of total failures is greater in vagotomy high-failure synapses**

<table>
<thead>
<tr>
<th>Stimulus Shock</th>
<th>Percent Total Failures of Control High Failure Synapses</th>
<th>Percent Total Failures of Vagotomized High Failure Synapses</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 ± 2.0</td>
<td>3 ± 2.0</td>
<td>0.7</td>
</tr>
<tr>
<td>2</td>
<td>10 ± 2.0</td>
<td>17 ± 4.0</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>22 ± 4.0</td>
<td>38 ± 6.0</td>
<td>0.03</td>
</tr>
<tr>
<td>4</td>
<td>29 ± 3.8</td>
<td>42 ± 5.6</td>
<td>0.07</td>
</tr>
<tr>
<td>5</td>
<td>29 ± 3.6</td>
<td>48 ± 4.6</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Following vagotomy, high-failure synapses have a greater percentage of total failures when compared with control high-failure synapses. Though the difference is only significant after the third and fifth shocks (during a 50-Hz stimulus train), vagotomized high-failure synapses clearly have more overall failures than control high-failure synapses (see Fig. 6C). Percent failures were derived after each stimulus by counting the total number of failures and dividing that number by 10 (for the 10 trials each cell underwent) multiplied by the total number of control high-failure ($n = 20$) or vagotomized high-failure ($n = 24$) synapses. Values are presented as means ± SE.
FIG. 7. Median initial eEPSC amplitude across treatment and failure groups. Although high-failure synapses have significantly smaller eEPSCs when compared with low-failure synapses ($P < 0.05$; Kruskal-Wallis ANOVA followed by Dunn’s pairwise multiple comparison procedure), there are no significant interactions between control and vagotomy low-failure synapses nor between control and vagotomy high-failure synapses ($P > 0.05$). Error bars represent 25–75% confidence interval. Asterisk (*) represents significance between control low failure and control high failure ($P < 0.05$), #, significance between vagotomy low failure and vagotomy high failure ($P < 0.05$).

unchanging spontaneous and miniature EPSC data suggest that the alterations due to vagotomy are likely to be presynaptic in nature.

**Discussion**

In the current work, we unilaterally sectioned a portion of the vagus nerve (vagotomy) in vivo to test whether vagotomy altered the efficacy of monosynaptic transmission in the NTS in vitro. Our results revealed that the amplitudes of monosynaptic eEPSCs were depressed following vagotomy. Based on prior studies indicating that NTS synapses undergo homeostatic plasticity in response to peripheral insults to vagal afferent axons, this outcome was unanticipated.

The theory of homeostatic plasticity predicts that long-term alterations to presynaptic activity will cause compensatory changes in the electrophysiological properties of the postsynaptic neuron, resulting in a return to the original set point of the system (Turrigiano and Nelson 2000). Previous studies have demonstrated that NTS neurons and their synapses can undergo homeostatic plasticity (reviewed by Bonham et al. 2006). We therefore anticipated that vagotomy, by eliminating the propagation of action potentials from the visceral organs to monosynaptic NTS neurons, would cause a homeostatic, compensatory increase in synaptic efficacy at this first synapse. Contrary to these expectations, we observed a significant decrease in synaptic efficacy characterized by a reduction in the median amplitude of monosynaptic eEPSCs. The vagotomy-induced reduction in synaptic efficacy appears to be presynaptic in origin because both the amplitudes of mEPSCs as well as the passive and active properties of postsynaptic NTS neurons were unaffected.

Are synaptic changes caused by the elimination of action potentials or by injury-induced modulators?

Although homeostatic theory would predict that synaptic efficacy would increase following vagotomy, a reduced synaptic response to axotomy is not unprecedented in peripheral nerve literature. Gallego et al. (1979) demonstrated that after 1 wk, axotomy of the medial gastrocnemius nerve of cats caused a decrease in the amplitude of evoked monosynaptic excitatory postsynaptic potentials (EPSPs) recorded from spinal motoneurons. This decreased EPSP amplitude could not be reversed by daily electrical stimulation of the intact central process of the axotomized nerve. On the other hand, chronic action potential blockade of the intact nerve with tetrodotoxin (TTX) caused a significant increase in the amplitude of EPSPs. These findings suggest there is an important difference between synaptic changes elicited by injury to a nerve and synaptic changes induced by the cessation of action potential conduction. Indeed, this premise is supported by recent work. Three days after IA afferent nerve crush, IA EPSPs recorded from rat spinal motoneurons significantly increased in amplitude. Although electrical stimulation proximal to the site of nerve crush could reverse the EPSP increase, the use of choline to block axonal transport revealed that the EPSP amplitude increase caused by nerve crush and subsequent reversal by electrical stimulation were not dependent on action potential activity. Instead, the authors suggested that the trafficking of an unknown molecular signal up an intact axon was the source of the increased EPSP amplitude (Bichler et al. 2007). It is therefore reasonable to suggest our results indicate that vagotomy, rather than simply blocking the transmission of vagal afferent impulses, may be eliciting a complicated, injury-induced response at NTS synapses. This suggestion is supported by previous work showing that following vagotomy, the hypox excitability observed in nodose neurons can be reversed by the chronic application of glial derived neurotrophic factor (GDNF) (Lancaster 2003) to the cut ends of vagal axons. In future experiments, it will be interesting to test whether GDNF application, or the application of other neurotrophic factors, can reverse vagotomy-induced synaptic depression.

**Low- versus high-failure synapses**

Our results demonstrated that the NTS of both control and vagotomized rats had two distinct populations of monosynaptic synapses: those that fail to produce an EPSC <5% of the time following 50-Hz stimulation of the ST and those that fail 5% of the time or more. The existence of two failure-type populations of synapses in the rat NTS is in accord with observations recently reported by Andresen and Peters (2008). Outside of the decreased amplitude of the initial eEPSC in high-failure synapses and the longer latency of high-failure synapses, all other tested electrophysiological parameters of these two synaptic populations were identical.

After vagotomy, the percentage of high-failure synapses significantly increased when compared with controls. The greater population of low-eEPSC amplitude, high-failure synapses following vagotomy resulted in an overall decrease in synaptic efficacy demonstrated by the significantly reduced initial eEPSC amplitude following vagotomy. It is important to note that although not significant, our results show a trend toward vagotomized high-failure synapses having an even smaller initial eEPSC than control high-failure synapses [−88 (−66 to −150) vs. −61 (−42 to −110) pA]. Additionally, our results uncovered that all synapses from vagotomized animals (undifferentiated by failure group) have a significantly larger overall failure rate at least double that of controls in response to all stimulus shocks excluding the first. This increase cannot be explained solely by the greater percentage of high-failure synapses following vagotomy, as vagotomized high-failure synapses have a
greater percentage of failures than control high-failure synapses.

Possible mechanisms for the vagotomy-induced shift to high-failure synapses: TTX sensitivity

Previously, Lancaster et al. (2000) demonstrated that 5–6 days following vagotomy the cell bodies of the vagus nerve (nodose ganglion neurons) become less excitable and require a higher rheobase current to elicit an action potential that control cell bodies. They argued that this was due, in part, to a decrease in the density of TTX-resistant sodium currents (TTX-R), currents important to the normal sustained action potential discharge nodose neurons experience in response to continued stimulation (Lancaster and Weinreich 2001). This reduction in TTX-R sodium currents leaves TTX-sensitive (TTX-S) currents the primary sodium current in vagotomized cell bodies. As a result of the decrease in TTX-R current following vagotomy, the inactivation curve for sodium conductance is shifted toward more hyperpolarized levels while the repriming of the sodium current is significantly slower when compared with control nodose neurons.

The pharmacological and electrophysiological properties of the cell bodies of peripheral sensory neurons are regularly used as an experimental model for the central nerve terminals, which often cannot be studied directly due to their small size and inaccessibility. If the sodium current properties of the nodose cell bodies can be extrapolated to the central vagal nerve terminals, it is possible that a reduced excitability of most central nerve terminals following vagotomy causes a significant decline in the number of times an action potential invades the terminal in response to repeated stimulation. Following vagotomy, Lancaster and Weinreich (2001) determined that the fraction of sodium currents in nodose neurons that were reprimed at 20 ms following stimulation were significantly reduced by \( \sim 10\% \). This decrease in the availability of reprimed sodium channels during a sustained or high-frequency stimulus contributed to the significant reduction in neuronal firing. In our experiments, the five pulse ST stimulation protocol had an interstimulus interval of 20 ms. It is plausible, therefore, that a decrease in available sodium channels, along with a more hyperpolarized inactivation profile, could lead to a greater percentage of synaptic failures through a decrease in the amount of neurotransmitter released from the under-excited nerve terminal. In addition, out of a population of normal nodose neurons, there are cells that express predominantly TTX-S sodium currents. These cells could conceivably account for the \( \sim 30\% \) of control synapses that have a high-failure rate.

Possible mechanisms for the shift to high-failure synapses: reorganization of myelinated axons

A recent study has classified low- and high-failure synapses within the normal NTS based on capsaicin sensitivity (Andersen and Peters 2008). In that study, low-failure synapses correspond to neurons receiving capsaicin-resistant, myelinated fibers, whereas high-failure synapses correspond to neurons receiving capsaicin-sensitive, unmyelinated afferents. Assuming the high-failure synapses in our study are capsaicin-sensitive, unmyelinated fibers while low-failure synapses are capsaicin-resistant and myelinated, we would like to suggest three possible explanations for the shift to larger numbers of high-failure synapses following vagotomy: 1) there could be a selective pruning of terminals arising from myelinated fibers, 2) there could be a sprouting of terminals rising from unmyelinated fibers, or 3) there could be a physiological change to the nerve terminals that leads to an increase in the percentage of high-failure synapses and overall failures. There is precedence for each of these scenarios.

After sciatic nerve section, there is anatomical and electrophysiological evidence for the reorganization of afferent nerve terminals based on fiber-type in the dorsal horn (but see Hughes et al. 2003). At least 2 wk following axotomy, HRP-labeled myelinated sensory axon terminals appear to sprout into lamina II of the dorsal horn, a layer in which they were previously absent (Woolf et al. 1995). Supporting these findings, cellular activity has been recorded from spinal cord dorsal horn neurons following axotomy of the sciatic nerve (Kohama et al. 2000; Okamoto et al. 2001). These results indicate that axotomy caused a reorganization of A\( \beta \) fiber terminals, increasing synaptic contacts within the dorsal horn. Conversely, Kohno et al. (2003) have demonstrated not only a sprouting of selective nerve terminals in the dorsal horn following axotomy but also a pruning of other terminals, specifically those arising from A\( \delta \) and C fibers. Assuming high-failure synapses are capsaicin-sensitive, our results would indicate the opposite scenario from the sprouting and pruning data of the somatosensory system; a possibility that is not unanticipated given the different embryological origins of the two systems (Baker 2005). Apart from the anatomical sprouting and pruning of nerve terminals following axotomy, there are data suggesting that the nerve terminals themselves can undergo physiological changes following peripheral sensory nerve inflammation and injury. Studies in the somatosensory system have shown that inflammation can cause A fibers to take on phenotypical features characteristic of C fibers (see review by Woolf and Costigan 1999). Relevantly, Zhang et al. (2008) demonstrated that myelin-ated vagal bronchopulmonary afferent axons that are normally minimally sensitive to capsaicin can become capsaicin-sensitive after chronic inflammation of the airway. Furthermore, the cell bodies of afferents coming from the lungs demonstrated an increase in TRPV1-positive immunoreactivity. Both of these studies demonstrate that sensory afferents are capable of changing their cellular phenotypes following injury and inflammation. Although it is presently unknown whether vagotomy primarily affects capsaicin-sensitive or -resistant synapses, future studies could differentiate between these two populations to investigate any anatomical or phenotypical changes caused by vagotomy.

Finally, it is important to note that although all the NTS neurons from which we recorded received direct synaptic connections with nerve terminals arising from the ST (indicated by the low-jitter, monosynaptic eEPSCs), we cannot be sure that every synapse received nerve terminals that were vagal in origin. The ST also contains axons arising from the glosopharyngeal and facial nerves. Although the exact percentage of axons contributed by each nerve to the ST is unknown, anatomical studies have shown that the vagus nerve is the primary contributor to the medial region of the NTS, from which we recorded, while the glosopharyngeal and facial
nerves tend to synapse more laterally (Contreras et al. 1982). Furthermore, the fact remains that following section of the vagus nerve, significant changes in the populations of high-failure synapses and overall synaptic failures occurred. The most parsimonious explanation for our findings would suggest that a significant number of the neurons from which we recorded were receiving inputs that were vagal in origin as it was the vagus nerve which we manipulated. Indeed if some of the NTS neurons recorded from had nonvagal sources, this possibility would suggest that our recordings might have underestimated the effects of vagotomy.

In summary, our results investigated the effects of unilateral vagotomy on monosynaptic communication in the NTS, a major component of the autonomic reflex circuit. These findings revealed that vagotomy causes a decrease in synaptic efficacy at second-order NTS synapses manifested by attenuated evEPSC amplitudes. This decrease is likely presynaptic in nature and produced by an increase in synaptic failures and high-failure synapses. Despite the rising use of vagal manipulations to treat medical pathologies, underlying neural changes are often underestimated the effects of vagotomy.