JNeurosci
THE JOURNAL OF NEUROSCIENCE

Research Articles: Systems/Circuits

Enhancing GABAergic Tone in the Rostral Nucleus of the Solitary Tract Reconfigures Sensorimotor Neural Activity

https://doi.org/10.1523/JNEUROSCI.0388-20.2020

Cite as: J. Neurosci 2020; 10.1523/JNEUROSCI.0388-20.2020

Received: 18 February 2020
Revised: 11 November 2020
Accepted: 15 November 2020

Enhancing GABAergic Tone in the Rostral Nucleus of the Solitary Tract Reconfigures Sensorimotor Neural Activity

Joshua D. Sammons[1], Caroline E. Bass[2], Jonathan D. Victor[3], and Patricia M. Di Lorenzo[1,4]

[1]Dept. of Psychology, Box 6000, Binghamton University, Binghamton, NY 13902-6000
[2]Dept. of Pharmacology and Toxicology, Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY 14203
[3]Feil Family Brain and Mind Research Institute, Weill Cornell Medical College, NY, NY 10065
[4]To whom correspondence should be addressed
Dept. of Psychology
Box 6000
Binghamton University
Binghamton. NY 13902-6000
Email: diloren@binghamton.edu

Abbreviated title: Enhanced GABA tone in rNTS modifies taste responses
Number of pages: 45
Number of figures: 12
Number of Tables: 2
Number of words in
Abstract: 250
Introduction: 646
Discussion: 1,500

Conflict of Interest: The authors declare no competing financial interests.

Acknowledgements: Supported by NIDCD Grant RO1-DC006914 to PMD.
J.D. Sammons' current affiliation is Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL 35294-2170

ABSTRACT

Recent work has shown that most cells in the rostral, gustatory portion of the nucleus tractus solitarius (rNTS) in awake, freely licking rats show lick-related firing. However, the relationship between taste-related and lick-related activity in rNTS remains unclear. Here, we tested if GABA-derived inhibitory activity regulates the balance of lick- and taste-driven neuronal activity. Combinatorial viral tools were used to restrict expression of ChR2-EYFP to GAD1+ GABAergic neurons. Viral infusions were bilateral in rNTS. A fiberoptic fiber attached to a bundle of drivable microwires was later implanted into the rNTS. After recovery, water-deprived rats were presented with taste stimuli in an experimental chamber. Trials were 5 consecutive taste licks [NaCl, KCl, NH_4Cl, sucrose, MSG/IMP, citric acid, quinine, or artificial saliva (AS)] separated by 5 AS rinse licks on a VR5 schedule. Each taste lick triggered a 1s train of laser light (25Hz; 473nm; 8-10mW) in a random half of the trials. In all, 113 cells were recorded in the rNTS, 50 responded to one or more taste stimuli without GABA enhancement. Selective changes in response magnitude (spike count) within cells shifted across-unit patterns but preserved inter-stimulus relationships. Cells where enhanced GABAergic tone increased lick coherence conveyed more information distinguishing basic taste qualities and different salts than other cells. In addition, GABA activation significantly amplified the amount of information that discriminated palatable vs. unpalatable tastants. By dynamically regulating lick coherence and remodeling the across-unit response patterns to taste, enhancing GABAergic tone in rNTS reconfigures the neural activity reflecting sensation and movement.

Significance Statement

The rostral nucleus tractus solitarius (rNTS) is the first structure in the central gustatory pathway. Electrophysiological recordings from the rNTS in awake, freely-licking animals show that cells in this area have lick- as well as taste-related activity, but the relationship between these characteristics is not well understood. Here, we showed evidence that GABA activation can dynamically regulate both of these properties in rNTS cells to enhance the information conveyed, especially about palatable vs. unpalatable tastants. These data provide insights into the role of inhibitory activity in the rNTS.

INTRODUCTION

In mammals, information about taste is conveyed directly to the rostral nucleus tractus solitarius (rNTS). This structure directs taste information to higher-order structures, integrates information from centrifugal sources and, ultimately, influences movements aimed at ingestion. In rNTS of alert rats, only a minority of cells are taste-responsive; most cells, including taste-responsive cells, track behavior (Denman et al., 2019). That is, when rats freely lick tastants of various qualities, coherence of firing patterns with the lick cycle is very common (Denman et al., 2019). The phase of the lick cycle associated with maximal firing varies widely from cell to cell, indicating that the lick-related responses are not simply motor signals or efference-copy. Moreover, lick-related cells, by the arrangement of their spikes over time, also contribute information about taste quality along with canonically taste-responsive cells, albeit at a lower level (Denman et al., 2019; Roussin et al., 2012; Weiss et al., 2014). Thus, there is an intimate relationship between taste sensation and the movements associated with ingestion in the rNTS.

The sensorimotor aspects of rNTS activity suggest that taste-responsive cells collaborate with behavior-driven cells to encode taste; however, the extent to which taste responses can be altered experimentally or physiologically reveals a surprising amount of plasticity. For example, taste responsivity within a cell can be altered by taste adaptation (Di Lorenzo and Lemon, 2000), differences in taste context (Di Lorenzo et al., 2003) or the simple passage of time (Sammons et al., 2016), even to the point where taste responses that were not previously evident were uncovered. Further, suppression (Monroe and Di Lorenzo, 1995) or stimulation (Smith and Li, 2000) of the gustatory cortex, lateral hypothalamus (Cho et al., 2002, 2003; Matsuo et al., 1984; Murzi et al., 1986) and amygdala (Cho et al., 2003; Li et al., 2002), all of which provide

centrifugal input to rNTS, can selectively alter responses to individual tastants in rNTS cells.

One potential mechanism that may underlie or contribute to these changes is the action of GABA, since several structures that send descending input to the rNTS either synapse on GABAergic interneurons (Smith and Li, 2000) or provide GABAergic input directly to rNTS neurons (Saha et al., 2002).

The presence of GABA in the rNTS has been well documented (Boxwell et al., 2013; Davis, 1993; Lasiter and Kachele, 1988), but the functional consequences for taste coding are not fully understood. Leonard et al. (1999) argued that the localization of GABAergic terminals on dendrites in rNTS facilitates modulation of incoming gustatory signals. In physiological studies, Grabauskas and Bradley (1998; 1999) showed that tetanic stimulation of the solitary tract induces both short- and long-term GABA-mediated potentiation of inhibitory synaptic activity, suggesting that this type of presynaptic plasticity may aid in stabilizing the response to afferent input (Grabauskas and Bradley, 1999). In addition to inhibition produced by afferent signals, taste-responsive cells in the rNTS are under tonic inhibitory influence (Grabauskas and Bradley, 2003; Smith and Li, 1993) presumably derived from GABAergic interneurons. Application of the GABA antagonist bicuculline can broaden the breadth of tuning of taste-responsive rNTS cells. Moreover, inhibitory interactions in rNTS may enhance and stabilize the temporal structure of taste-evoked spike trains (Rosen and Di Lorenzo, 2009). The caveat to what is known about GABA-driven inhibition in rNTS is that it is all derived from studies in anesthetized subjects; the function of inhibition in taste coding in awake subjects may be different.

Here, we tested the hypothesis that inhibition in the rNTS can modulate both the sensory and behavior-related activity in rNTS to alter taste coding. We used optogenetic tools to selectively enhance GABAergic activity in rNTS while rats freely licked taste stimuli. Results showed that

GABA activation can selectively modify taste responses and can modulate lick coherence in a subset of cells. These changes reconfigured the relationship of sensory to motor-related activity in these cells, enhancing the information they conveyed about taste.

MATERIALS AND METHODS

Subjects

Six male (250-450 g) and three female (200-350 g) Sprague-Dawley rats obtained from Taconic Laboratories (Germantown, New York) served as subjects. Of these, two males and two females served as non-viral control subjects. Food and water were provided *ad libitum* except during behavioral studies where rats were water deprived for 22-23 h per day. Rats were pair-housed and maintained on a 12 h light-dark cycle with lights on at 2100 hours. All procedures were approved by the Institutional Animal Care and Use Committee of Binghamton University and conducted in accordance with the National Institutes of Health Animal Welfare Guide.

Viral constructs and infusion

Rats were anesthetized with a ketamine:xylazine mixture (100 mg/kg:14 mg/kg, i.p.). Buprenorphine-HCl (0.05 mg, s.c.) was administered to enhance the effects of the anesthetic and atropine sulfate (0.054 mg/kg, s.c.) to prevent excessive secretions. The rat's scalp was shaved and its head was secured in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA). The head was leveled with bregma and lambda in the same dorsal-ventral plane. The rat's eyes were lubricated and core temperature maintained at 37 °C with a heating pad attached to an anal thermistor probe. The scalp was then swabbed three times with Betadine alternated with 70%

ethanol. An incision was made along the midline from bregma to the occipital ridge and the skin and fascia were retracted with blunt dissection. A hole was drilled at 12 mm posterior and ±1.75 mm lateral to bregma. A combination of viruses was infused (0.5 µL total; 0.5 µL/min) bilaterally 6 mm below the surface of the brain. The combination consisted of 166 nL of GAD1-Cre-AAV 2/10 + 333 nL of Ef1α-DIO-ChR2-EYFP-AAV 2/10, which we have previously shown to restrict expression to GAD1+ neurons (Xiao et al., 1998; Wakabayashi et al., 2019). All viruses were packaged using the triple transfection method to generate pseudotyped virus as detailed elsewhere (Gompf et al. 2015). After each infusion, the needle was held in place for an additional 5 minutes to ensure complete expulsion of the virus. After retraction of the needle, the scalp was sutured and the rat allowed to regain consciousness. The animal was given a post-operative injection of buprenorphine-HCl (0.05 mg; s.c.) and gentamicin (0.05 mg; s.c.). Rats were allowed to recover for 2-4 wk. Non-viral control rats (n=4; two male, two female) experienced the same surgical procedures as experimental rats but without viral infusion.

Optrode implantation surgery

Two to four weeks after viral infusion surgery, optrodes were implanted into the rNTS. Initially, rats were given buprenorphine-HCl (0.05 mg; s.c.) and atropine sulfate (0.054 mg/kg; s.c.). Animals were then anesthetized with 3% isoflurane in O_2 at a flowrate of 0.9 L/min and the scalp was shaved. Anesthesia was maintained with 1-3% isoflurane. The rat's head was placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA) and swabbed with betadine and 70% ethanol 3 times. The eyes were lubricated and the rat's temperature was maintained at 37 °C throughout the surgery. The skull was exposed from just anterior to bregma to about 1.5 cm behind the occipital ridge. Five self-tapping screws were inserted into the skull. The head was

angled with bregma 4 mm below lambda and a hole drilled at 14.3-15.3 mm posterior and 1.7-1.8 mm lateral to bregma. The exposed dura was resected and an optrode consisting of 8 or 16 tungsten wires attached to a fiberoptic implant were lowered through the hole to ~ 5-6 mm below the surface of the brain at a rate of 1mm per 5min. The lower tip of the fiberoptic implant was positioned within 100μm of the tip of the microelectrode bundle. This arrangement ensured that the light stimulation impacted the neurons that were recorded (Yizar et al. 2011). The 16 channel electrode + fiberoptic bundles were drivable and placed ~ 500 μm above the rNTS. A ground wire was wrapped around one of the skull screws. The entire assembly was then embedded in dental acrylic. Rats were administered buprenorphine-HCl (0.05 mg; s.c.) and gentamicin (0.05 mg; s.c.) immediately following surgery and daily for two additional days. The rat was allowed to recover for 5 days or until it regained 90% pre-surgical body weight before testing began.

Apparatus

For an experimental session, rats were placed in an operant chamber (Med Associates, St. Albans, VT) housed in an MDF outer box equipped with a house light and fan. One wall of the operant chamber had an opening that allowed access to a lick spout for delivery of taste stimuli. The occurrence of a lick was detected when the rat broke an infrared beam as it accessed the lick spout. The stainless steel lick spout housed a collection of 16 stainless steel tubes for delivery of 16 different taste stimuli. Reservoirs of taste stimuli were pressurized with air (~10 psi). Polyethylene (PE) tubing connected the stimulus reservoirs to solenoids that, when activated by a computer signal, delivered ~12 μL of fluid to the lick spout through PE tubing attached to the stainless steel tubes in the lick spout.

183

184 ***Experimental Paradigm***

185 Rats were moderately water deprived (22-23h) and placed in the operant chamber where they
186 had free access to the lick spout for the entire experimental session (30 min). Taste stimuli
187 consisted of 0.1 M sucrose, 0.1 M NaCl, 0.1 M monosodium glutamate (MSG) plus 0.01 M
188 inosine monophosphate, 0.1 M KCl, 0.1 M NH_4Cl, 0.01 M citric acid, 0.0001M quinine, and
189 artificial saliva (AS; 0.015 M NaCl, 0.022 M KCl, 0.003 M $CaCl_2$; 0.0006 M $MgCl_2$; pH ~ 7.4;
190 Hirata et al., 2005; Breza et al., 2010). All tastants were reagent grade and dissolved in AS. (AS
191 was presented as both a rinse and a taste stimulus.) The order of taste stimulus presentations was
192 randomized. There were two types of licks: reinforced and dry. Each reinforced lick delivered
193 ~12 µL of fluid. A taste trial consisted of five consecutive reinforced licks of a taste stimulus
194 with no intervening dry licks. Between trials, five licks of an AS rinse were presented on a
195 variable ratio 5 (VR5) schedule, with each reinforced AS lick occurring every 4-6 dry licks.
196 During a randomly interspersed half of the taste stimulus trials, laser stimulation of GABAergic
197 neurons (473 nm; 25 Hz; 10-12 mW) was triggered for 1s after each reinforced stimulus lick.
198 Fig. 1A shows a schematic of a typical sequence of taste stimulus trials (5 consecutive reinforced
199 licks) interspersed with rinse licks (presented on a VR5). Fig. 1B shows a sequence of licks over
200 30 s from an actual test session. The fiberoptic implant was static, but every 2-4 recording days,
201 the microwires were extended ventrally 25-50 µm. Experimental sessions were 30min in length
202 and continued daily, except for weekends, for 2-4 wks.

203 - - - - - - - -

204 Insert Fig. 1 about here.

205 - - - - - - - -

206

207 ***Electrophysiological Recording and Light Stimulation***

208 During the experimental session, the rat's electrode bundle was connected to an Omniplex D
209 Neural Data Acquisition System (Plexon, Dallas, TX). Timing for electrophysiological activity
210 and stimulus events were recorded using PlexControl software (Plexon, Dallas, Tx). The
211 fiberoptic implant was attached to a 473 nm laser source (Shanghai Laser and Optics Century
212 Co., Ltd., Shanghai, China) through a fiberoptic patch cable (1m length, 200µm core, 0.22 NA)
213 (THORLABS, Newton, NJ). Optic stimulation was triggered in a random half of tastant trials as
214 mentioned in the experimental paradigm section.

215 Neuronal signals were isolated in Offline Sorter (Plexon, Dallas, TX) or through a semi-
216 supervised spike sorting Python program adapted from Mukherjee et al. (2017)
217 (https://github.com/dmarshall-bing/AutoSort). Less than 0.5% of waveforms contained an
218 interspike interval less than 1 ms.

219

220 ***Analyses of Taste Responses***

221 Spontaneous firing rate was calculated as follows: First, periods where the rat was not licking
222 for at least 10 s were identified. Next, the first 3 s and last 1 s of activity during that period were
223 discarded to ensure that the remnants of a lick bout or preparation for a lick bout were not
224 included as spontaneous activity. Finally, firing rates during these periods without licking were
225 pooled, divided into 1 s intervals and the overall firing rate calculated in spikes per s (sps).

226 As in previous work (Escanilla et al., 2015; Roussin et al., 2012; Weiss et al., 2014),
227 responses to taste stimuli were detected over two time scales: 1) responses that extended across

more than one lick, called "5-lick" responses, and 2) responses that occurred briefly after each lick, called "lick-by-lick" responses.

5-lick responses were quantified by a significant increase or decrease in firing rate over five consecutive taste licks (without intervening un-reinforced licks), compared to baseline firing rate for at least 300 ms. Baseline firing rate was calculated in 100 ms time bins over the 1s preceding the first taste stimulus lick in a trial. To determine if a significant response was present, the firing rate in 100 ms time bins, beginning with the first taste stimulus lick, was compared to the 95% confidence limits of the baseline firing rate. The 100 ms window was moved in 20 ms increments until there were at least three consecutive, non-overlapping 100 ms bins where there was a significant difference between baseline and response firing rates. The leading and trailing edge of the significant bins were used to determine when a taste response started (latency) and ended (duration) respectively. A maximum of two bins within a response were allowed to be non-significant. The response magnitude (firing rate during a response minus the baseline firing rate), latency, duration, and baseline activity were calculated for each taste response. Neurons with a response spike rate less than 2 spikes per sec (sps) were not included.

Lick-by-lick responses were detected using a Chi-squared test comparing responses from the average spike rate of the last non-reinforced lick (i.e. a dry lick) before every tastant trial to the average spike rate of every lick from each tastant. Response windows were limited to 150 ms after each lick and divided into ten 15 ms bins. We chose the interval of 150 ms to measure lick-by-lick responses because this was the median interlick interval overall. We chose 15 ms bins because not every lick-by-lick response spanned the full 150 ms. Had we just used the entire 150 ms interlick interval to measure the responses, we might have missed some very brief but significant responses that occurred following each lick of a taste stimulus. Using the chi-square

test, the actual response value from each bin of each tastant vs dry lick was compared to the corresponding expected response bin. A Bonferroni correction was made for multiple (n = 16 tastants, i.e. 8 tastants with and without GABA activation) comparisons. Neurons with a response firing rate less than 4sps during lick bouts were excluded.

To test for effects of GABA stimulation on taste responses, we performed a chi-square analysis of the peri-stimulus time histograms (PSTH; 100 ms bins from t = 0 to t = 4 s following the first taste stimulus lick) and compared GABA vs non-GABA stimulation to obtain a p-value. We then corrected for multiple comparisons using the false discovery rate method (FDR; Benjamini and Hochberg 1995).

Analysis of breadth of tuning

In addition to noting the number of tastants to which a cell responds, breadth of tuning was assessed by calculating two standard measures of tuning breadth that are more graded: taste entropy and taste sharpness. Each measure reflects a different aspect of tastant specificity. Both analyses were performed using the five prototypical tastants (sucrose, NaCl, MSG, citric acid, and quinine). Taste entropy (Smith and Travers, 1979) is a measure of uncertainty based on the similarity of response magnitudes between tastants. This measure is calculated as follows:

$$H = -k\sum_{i=1}^{n} P_i log P_i$$

where n is the number of tastants (5), k = 1.4307 for 5 tastants, and P_i is the ratio of tastant i response magnitude to the sum of all tastant response magnitudes. The value ranges from zero, signifying that the neuron responds to a single tastant, to one indicating that the neuron responds

to all 5 tastants equally. Taste sharpness (Rainer et al., 1998) is a measure of how similar taste magnitudes are to the best stimulus and is calculated as follows:

$$Sharpness = \frac{(n - \sum_{i=1}^{n} T_i/T_{best})}{n-1}$$

where n is the number of tastants, T_i is response magnitude for tastant i, and T_{best} is the response magnitude of the best stimulus. Similar to the entropy measure, a value of zero indicates a response to a single tastant, a value of one indicates equal responses to all five tastants.

Temporal Coding Analysis

Analysis of information about taste quality conveyed by individual neurons was performed using metric space analyses (MSA; Victor and Purpura 1996, 1997). This method has been described in detail previously (Roussin et al. 2012; Weiss et al. 2014; Escanilla et al. 2015; Sammons et al. 2016) and is only summarized here. The basic approach of MSA is to measure the "cost" of converting one spike train, e.g. a response to a tastant, into another as a measure of similarity/dissimilarity. Cost is accrued by insertion or deletion of spikes or movement of spikes in time. The insertion or deletion of a spike costs one arbitrary unit. Movement of a spike in time costs qt units where q is a parameter of temporal precision ($1/q$ has units of seconds) and t is the amount of time that the spike is shifted. Thus, at $q = 0$, the cost of moving a spike is zero, so spike timing is ignored when comparing spike trains; as q increases, spike timing is taken into account with progressively greater precision. At each value of q, the mutual information H between tastants and neural responses is estimated by comparing the similarity of pairs of responses to the same stimulus with the similarity of responses to different stimuli. To mitigate

biases due to sample size, the Treves-Panzeri-Miller-Carlton (TPMC) debiaser was applied to all estimates of H (for a review see Panzeri et al. 2007). This computation of information conveyed about taste quality is carried out across a range of values of q, and the maximum is denoted H_{max}.

Two auxiliary analyses using synthetic data were also conducted. First, to account for residual bias in the estimation of information, spike trains for 40 pairs of randomly-labeled responses were compared using MSA; this yields $H_{shuffled}$. Second, to determine whether temporal information was due to spike timing *per se*, vs. differences in the rate envelope, spikes within each taste-evoked spike trains were randomly assigned to alternative responses to the same tastant, while preserving the rate envelope; calculation of information from these synthetic datasets this yields $H_{exchange}$. Information about taste quality conveyed by spike timing was considered significant only if $H_{max} > H_{shuffled}$+2SD and $H_{max} > H_{exchange}$. If $H_{max} > H_{shuffled}$+2SD but not $H_{exchange}$, information was considered significant, but information conveyed by spike timing was not considered significant. Information from neurons where $H_{max} \leq H_{shuffled}$+2SD was set to zero.

To characterize the information conveyed by the population of cells, we calculated the average amount of information conveyed by the entire sample of units at 200, 500, 1000, 1500, and 2000 ms of the cumulative response. Information conveyed by the lick pattern was determined in the same way as for spike trains, and compared with that conveyed by spike trains. Only neurons from sessions that contained at least six taste trials with and six taste trials without laser stimulation were included in the temporal coding analysis.

Statistical Analyses of Lick Coherence

For each neuron's firing pattern, its coherence with the occurrence of licks was calculated using the NeuroExplorer 5.201 Coherence Analysis function (NexTechnologies, Colorado Springs, CO). Single taper Hann windowing was used to calculate the values of 256 frequency bins between 0 and 50 Hz frequency with a 50% overlap between windows. The analysis calculates confidence as described in (Kattla and Lowery, 2010). Neurons with a coherence value above 99% confidence between 4-9 Hz were considered lick-coherent. In lick-coherent neurons, differences in lick coherence were obtained around tastant licks with laser stimulation versus tastant licks without laser stimulation. The reported difference in coherence value was calculated as the maximum difference in coherence between 4-9 Hz. An F-test was used to determine whether the change in coherence observed between baseline conditions without GABA activation and during GABA activation was actually due to GABAergic activation, or random chance.

Spearman's rank correlation coefficient (ρ) was calculated to determine correlations between lick coherence and measures of taste specificity. The 2-tailed p-value for each value was obtained for each correlation and a Bonferroni correction was made for multiple (n = 6) comparisons. The six different comparisons were lick coherence versus taste tuning, taste entropy, and taste sharpness, each with and without GABA stimulation.

Histology/ Immunolabeling

Rats were euthanized with sodium-pentobarbital (390 mg/kg; i.p.). Just before expiration, 10 s of 1 mA DC current was passed through the microwire with the last taste response. The rat was then transcardially perfused with isosaline followed by 4% paraformaldehyde (PFA) in phosphate buffered saline (1x PBS). The brain was extracted and placed in 4% PFA overnight.

The next day, brains were washed 3 times with PBS and stored in 20% sucrose in 1x PBS. Brains were then sectioned into 35 µm coronal slices. Every other section was individually placed into wells of a 96 well dish containing a cryoprotectant (30% Ethylene Glycol, 30% Glycerol, 11.4 mM NaH_2PO_4-H_2O, and 38.4 mM Na_2HPO_4). The other half of the sections were placed directly onto superfrost plus slides and stained with cresyl violet for lesion site identification. The center of each lesion was taken as the final site of recording.

Sections placed into the cryoprotectant were removed and washed 3 times with 1x PBS. They were placed in blocking agent (10% bovine serum albumin (BSA), 0.1% Triton X, 1x PBS) and gently rocked for 1h at room temperature (RT). Sections were then placed in primary (10% BSA, 1:1,000 Rabbit anti-GFP (Abcam, Cambridge, UK, cat#AB290), 1:500 Mouse anti-NeuN (Millipore, Burlington, MA, cat# MAB377), 1x PBS) for an additional 2h at RT or overnight at 4°C. Sections were washed 3 times with 1x PBS and placed in secondary (1:500 AF488 conjugated Goat anti-Rabbit (Abcam, Cambridge, UK, Cat# AB150077), 1:500 Cy3 conjugated Donkey anti-mouse (Jackson Immuno Research Labs, West Grove, PA, cat# 715-165-151), 1:10,000 DAPI stain (Millipore, Burlington, MA, cat# 5.08741.0001), 1x PBS) for 1h at RT.

RESULTS

General response characteristics

We recorded 113 isolated neurons from the rNTS of five freely licking rats (four male and one female) with optrode implants. Without GABA stimulation, a total of 50 (of 113; 44%) neurons responded to at least one of the eight taste stimuli tested. With GABA stimulation, 43 (of 113; 38%) neurons responded to at least one of the eight taste stimuli. Four neurons were

unresponsive without GABA stimulation but showed taste responses with GABA stimulation resulting in a total of 54 neurons that responded to at least one tastant either with or without GABA activation. Of the 54 recorded taste neurons, 52 (96%) responded to the five prototypical tastants (sucrose, NaCl, MSG, citric acid, or quinine) while two only responded with inhibitory lick-by-lick responses to artificial saliva with GABA stimulation.

In all, there was no effect of laser stimulation in these non-viral control animals. There were 26 neurons, 15 of these taste-responsive, that were recorded in four non-viral control animals (two male, two female). In addition, there were 10 recorded channels in these animals that contained evidence of several cellular waveforms above the noise level that could not be isolated as single units but collectively showed taste responses but no effect of laser.

The average spontaneous firing rate for the population was 18.4 ± 2.8 sps, median = 5.6 sps. The spontaneous firing rate for taste responsive neurons (mean = 16.5 ± 4.3 sps, median = 5.2 sps) was not significantly different from the spontaneous firing rate for non-taste neurons (mean = 20.1 ± 3.7 sps, median = 7.0 sps). When animals began licking, the overall firing rate decreased for 19 (17%) of the neurons and increased for 39 (35%) of the neurons, regardless of whether the licks were reinforced or not.

Analyses of licking behavior

To assess the potential effect(s) of GABAergic stimulation on licking behavior, we examined the microstructure of licking for each taste stimulus with and without laser stimulation. Table 1 shows the results of those analyses. Median interlick intervals, measured during the 5-lick taste trial did not significantly differ when the taste-reinforced licks were presented with or without GABA stimulation (Wilcoxon signed-rank test, $p = 0.188$). Moreover, there was no

significant difference in the median number of pauses during the 5-lick taste trial (Wilcoxon signed-rank test, $p = 0.336$) and no significant difference in the pause length (Wilcoxon signed-rank test, $p = 0.453$). Finally, there was no significant difference in the total time to complete the 5-lick taste trials across tastants (Wilcoxon signed-rank test, $p = 0.945$). In all, these results suggest that GABA activation during taste acquisition did not alter lick patterns per se.

- - - - - - - - -

Insert Table 1 about here.

- - - - - - - - -

GABAergic stimulation changed taste profiles of individual neurons

Fig. 2, illustrating taste responses from six different cells with and without GABA activation, shows that GABA activation selectively modified taste response magnitudes in 22 (of 54, 41%) rNTS cells. GABA stimulation sometimes enhanced responses (Fig. 2A, B), even when there were no responses without GABA activation. Conversely, activation of GABA attenuated or eliminated responses to taste stimuli at other times (Fig. 2A, B). There were 10 occasions where GABA activation enhanced some stimuli and attenuated others in the same cell. Fig. 2C shows the responses of four different cells illustrating the lack of an effect of the laser in non-viral control animals.

- - - - - - - - - -

Insert Fig. 2 about here.

- - - - - - - - - -

Taste response magnitudes with and without GABA activation for all taste responsive cells are shown in Fig. 3; both enhancement and attenuation of taste responses by GABA were evident

409 across cells. Stimulation of GABA release in the rNTS changed taste response magnitudes for
410 both 5-lick (Fig. 3A) and lick-by-lick responses (Fig. 3B). Neurons in this figure were organized
411 according to their best stimulus, i.e. the stimulus that evoked the largest response without GABA
412 stimulation (grey bars). Responses to tastants with GABA stimulation are overlaid as diamonds.
413 Table 2 summarizes the stimulus-by-stimulus effects of GABA activation on rNTS cells.
414 - - - - - - - - - -
415 Insert Fig. 3 and Table 2 about here.
416 - - - - - - - - - -
417 Based on previous studies that have suggested a role for GABA in modulating the breadth of
418 tuning in brainstem taste-responsive cells (Smith and Li,1998; Smith et al., 1998), we analyzed
419 the effects of GABA enhancement in rNTS cells using three complementary approaches. First,
420 we examined the number of tastants, each representing a basic taste quality, to which rNTS
421 responded before and after GABA activation. Results showed that GABA enhancement reduced
422 the number of tastants to which a neuron responded, consistent with previous reports in the
423 literature (Smith and Li, 1998). This is illustrated in Fig. 4. Although the total number of
424 responses to any given tastant was not altered by GABA stimulation (Chi-square = 1.46, $df = 4$, p
425 = 0.835), the number of tastants to which individual neurons responded decreased significantly
426 (Chi-square = 13.62, $df = 5$, $p = 0.018$). This was largely due to an increase in the number of
427 cells that were rendered unresponsive or only responded to a single stimulus with GABA
428 activation. Our second approach was the Uncertainty measure (Smith and Travers, 1979) which
429 did not show a significant difference with or without GABA activation. Specifically, the average
430 taste Uncertainty was 0.37 ± 0.05 without GABA stimulation and 0.48 ± 0.05 (Student's t test, p
431 = 0.535) with GABA stimulation. Finally, our third approach was the Taste Sharpness measure,

which also did not differ significantly following GABA activation. Average taste sharpness was 0.81 ± 0.03 without GABA stimulation and 0.76 ± 0.03 with GABA stimulation (Student's *t* test, $p = 0.698$). In sum, results show that GABA activation reduced the number of tastants to which a subset of units responded, resulting in changes in the breadth of tuning; however, across the population, there was no net effect of GABA activation on taste tuning (measured by the Uncertainty and Sharpness measures). Essentially, responses to various taste stimuli were redistributed across the population.

- - - - - - - -

Insert Fig. 4 about here.

- - - - - - - -

Effect of GABA on across-unit patterns

To determine whether GABA activation altered the pattern of responses to the tastants at the population level, we applied a multidimensional scaling analysis using Pearson correlations as measures of similarity. A hypothetical "taste space" placed the across-unit patterns for each taste stimulus close together or far apart depending on their similarity/dissimilarity. Across-unit response patterns both before and during GABA activation were analyzed and graphed together. Fig. 5 shows the results of the combined analysis. Without GABA activation, response patterns to each of the five basic were well separated in taste space, suggesting that each tastant evoked easily discriminable patterns of response. With GABA activation, the configuration of across unit patterns was similar but shifted in space, indicating that the basic interrelationships among tastant-evoked response patterns was intact, but the identities of the units that contributed most to the pattern were different.

- - - - - - - - -

Inset Fig. 5 about here.

- - - - - - - - -

GABA alters lick coherence, especially in taste neurons

As coherence with licking is a prominent aspect of firing patterns in the NTS and points to integration of sensory and motor activity, we next asked whether this coherence is modulated by GABA. In our sample, the majority of rNTS neurons (97 of 113; 86%) were coherent with licking. Coherence values associated with all licking within a session will be termed overall lick coherence. In general, overall lick coherence values for taste neurons were significantly higher than those of non-taste neurons ($p < 0.001$; taste neurons: mean = $2.2*10^{-1}$, median=$1.7*10^{-1}$, n = 54; non-taste neurons: mean = $6.3*10^{-2}$, median=$2.4*10^{-2}$, n = 59).

To determine whether and how GABA activation affected lick coherence, we restricted coherence analysis to licks that resulted in taste stimulus delivery, since this is when GABA release was triggered. Coherence values associated with licking only during tastant delivery will be termed tastant-restricted lick coherence. Not surprisingly, tastant-restricted lick coherence values without GABAergic stimulation were also higher in taste neurons than in non-taste neurons ($p <0.001$; taste neurons: mean = $3.3*10^{-2}$, median = $2.3*10^{-2}$; non-taste neurons: mean = $1.0*10^{-2}$, median=$5.2*10^{-3}$). The distribution of lick coherence values for both the global lick coherence and tastant-restricted lick coherence can be seen on the abscissas of Figs. 6A and 6B, respectively. Figure 6B additionally tracks the change in tastant-restricted lick coherence upon GABAergic stimulation over the ordinate and shows that taste responsive neurons are affected to a greater degree than non-taste neurons.

- - - - - - - - -

Insert Fig. 6 about here.

478 - - - - - - - - -

479

480 ***GABA activation increased gustatory information in rNTS neurons***

481 To analyze the effect of enhancing GABAergic tone on temporal coding of taste stimuli, we
482 applied MSA to datasets with at least six repetitions of each tastant (with and without GABA
483 activation). Sixty neurons (38 taste-responsive; 22 non-taste-responsive) were included in these
484 analyses; as previously noted (Denman et al., 2019), neurons that are not considered "taste-
485 responsive" by classical criteria nevertheless may carry information about taste when analyzed
486 by MSA. That is, some aspect of their firing patterns, e.g. lick-relatedness, may convey
487 information about taste quality identity, even if overall firing rate does not have a detectable
488 dependence on tastant.

489 Among the 38 taste-responsive neurons, GABA stimulation reduced taste-related information
490 to zero in 10 and generated significant taste-related information in 14 (Fig. 7A). GABA
491 activation eliminated taste-related information from nine of the 22 non-taste-responsive neurons
492 and generated information from five non-taste-responsive neurons (Fig. 7B). (Note that, as
493 detailed in Methods, when the information conveyed about taste quality in a given neuron was
494 not significantly different than that in the randomly shuffled control, we set "information" at
495 zero.)

496 - - - - - - - - -

497 Insert Fig. 7 about here.

498 - - - - - - - - -

499 Taste-related information conveyed by spike timing was also analyzed at various response
500 intervals ranging between 200 ms to 2 s. Fig. 8 shows the results of those analyses. At 2 s,

GABA activation increased taste-related information on average by 0.11 bits (48% increase) when all cells with 5-lick taste responses were considered. To determine the relationship of these changes in information to changes in lick coherence, we divided all the neurons (n = 113) into quartiles based on the change in GABA-evoked changes in taste-restricted lick coherence. For the 5-lick taste neurons (n=27), 8 fell into the "decrease coherence" group, 11 fell into the "increase coherence" group, and 8 fell into the "no change in coherence" group (middle two quartiles). For the lick-by-lick taste neurons (n = 41), 17 decreased coherence with GABA stimulation, 13 increased coherence, and 11 had no change in coherence upon GABA stimulation.

Cells in the uppermost quartile in which GABAergic stimulation increased lick coherence (n = 24) had a consistent increase in taste information with a maximum increase of 0.16 bits (68% increase) at 2 s with GABA activation. Taste-related information was not affected by GABA stimulation in neurons in the bottom three quartiles. Thus, the increase in taste-related information associated with GABA activation was primarily carried by the neurons whose lick-related activity was most increased by GABA. Further, information on the rats' lick patterns of different taste qualities was overall slightly decreased by GABA activation, suggesting that changes in the lick pattern *per se* cannot account for the increased taste quality information.

- - - - - - - - -

Insert Fig. 8 about here.

- - - - - - - - -

Information about salty tastants is increased after GABAergic stimulation

In addition to the five prototypical tastants, we also tested KCl and NH_4Cl to determine if GABA stimulation would increase the distinction between tastants of the same taste quality. Over the entire population of neurons, GABAergic stimulation had no effect on information relayed on the salty tastants (Fig. 9). However, when broken into the effect of GABAergic stimulation on lick coherence, similar to information about taste qualities, information relayed about the salty tastants was increased when lick coherence was also increased (Fig. 9) with a maximum increase of 0.13 bits (51%) at 1.5 s. Similar to taste quality analysis, GABAergic stimulation had no effect on neurons with decreased coherence or no change in coherence and it had no effect on information from the lick pattern.

- - - - - - - - -

Insert Fig. 9 about here.

- - - - - - - - -

Effect of GABA activation on information about palatability

Much of the information increase obtained by GABA stimulation occurs a second or two after tastant delivery is initiated. This time epoch is thought to signal taste palatability, at least in the gustatory cortex (Katz et al. 2001). As such, we sought to determine if GABAergic stimulation would increase information about the palatability of tastants. We collapsed responses to sucrose and NaCl as the palatable tastants and collapsed responses to citric acid and quinine as the non-palatable tastants and performed MSA on the two groups. Once again, GABAergic stimulation increased information in the later time points (Fig. 10) with a maximum increase of 0.21 bits (118%) at 1.5 s. GABA-induced increase in palatability-related information was observed whether GABA activation increased lick coherence (maximum 0.27 (246%) at 1.5s),

546 decreased lick coherence (0.11 (58%) at 1.5s) or had no effect on lick coherence (0.23 (86%) at
547 1.5s), with a non-significant trend to greater increases in palatability-related information in those
548 neurons in which GABA had a larger effect on lick coherence. Again, information conveyed
549 solely by the lick pattern was not changed with GABAergic stimulation.
550 - - - - - - - - -
551 Insert Fig. 10 about here.
552 - - - - - - - - -
553
554 ***Histology***
555 Lesion analysis show that the electrodes were dispersed throughout the rNTS from 11.76 to
556 12.48. Fig. 11 shows the locations (asterisks) of the electrodes in each of the five rats from
557 which data were collected. As is apparent, the lesions were mostly lateral with the most rostral
558 lesion also being the most medial (Fig. 11A). Figure 11B shows channelrhodopsin expression in
559 the area surrounding the rNTS lesion. While there were many labeled neurons dorsal to the
560 rNTS, the proximity of the fiberoptic implant to the recording electrode ensured that these extra-
561 rNTS cells were not optogenetically activated (Yizar et al. 2011). In addition, the labeled area
562 dorsal to the rNTS is part of the vestibular nucleus, an area that is not known to send projections
563 to the rNTS.
564 - - - - - - - - -
565 Insert Fig. 11 about here.
566 - - - - - - - - -
567
568
569 **DISCUSSION**

570

571 Enhanced GABAergic tone in the rNTS remodeled the across-neuron pattern of taste
572 responsiveness and enhanced the information about taste quality and palatability conveyed by the
573 temporal characteristics of the response in a subset of ells. In general, there was a great deal of
574 diversity in the effects of GABA activation at the single cell level. In individual cells in the rNTS
575 taste response profiles were changed by laser stimulation of GABA terminals in about half (22 of
576 54; 47%) of the sample of neurons. Responses to some stimuli were enhanced and others
577 attenuated, sometimes within the same cell. In fact, there were neurons that responded to taste
578 stimuli only during GABAergic stimulation (n = 2) and others that were rendered completely
579 unresponsive to taste under GABAergic influence (n = 13). These GABA-induced cell-by-cell
580 changes did not shift the overall interrelationship among response patterns but instead changed
581 the identities of the cells that contributed to the across unit patterns of response associated with
582 each taste stimulus. Enhancing GABA did, however, change the temporal patterns of taste-
583 evoked activity such that the information discriminating palatable (sucrose and NaCl) vs.
584 unpalatable (citric acid and quinine) increased when longer (1-2s) taste responses were
585 considered.

586 Present results expand the results reported by Smith and Li (1998). In that study, either
587 GABA or the GABA antagonist bicuculline methiodide (BICM) was infused directly into the
588 rNTS in urethane-anesthetized hamsters. Their data suggested that GABA narrows the tuning of
589 taste-responsive cells. In awake rats in the present study, we confirmed that optogenetic
590 activation of GABA in rNTS narrows taste tuning in a subset of cells; however, it also broadened
591 the response profile in another subset of cells. While Smith and Li (1998) only tested the best
592 and second best stimulus with GABA and BICM, we tested the effects of GABA enhancement

593 for all of the basic tastants and found more complex effects. So, for example, it was not
594 uncommon for GABA enhancement to attenuate the response to one stimulus and amplify the
595 response to another in the same cell. In those cases, the breadth of tuning did not show a net
596 change, though the complement of tastants that evoked a response was altered.
597 Results of the MDS analyses illustrate the effect of augmenting GABAergic tone on
598 population coding of taste in rNTS. Specifically, under the influence of enhanced GABA release,
599 the configuration of the taste space generated by the across-unit pattern was essentially
600 unchanged compared to the taste space without GABA enhancement. That is, in both taste
601 spaces, patterns associated with the five basic taste qualities were well separated from each other.
602 However, the placement of taste stimuli in the taste space with GABA enhancement was
603 systematically shifted. This result implies that, for any given tastant, the identity of the cells that
604 conveyed the signal was shifted by GABA enhancement but the overall relationship between
605 taste qualities, as signaled by the across-unit pattern, was essentially unchanged.
606 While GABA enhancement affected taste response magnitudes, it also modified the temporal
607 arrangement of spikes within taste responses. Furthermore, these effects were correlated with
608 GABA-induced changes in lick coherence. In general, GABA enhancement boosted the
609 information conveyed about the five basic taste qualities. A closer analysis suggested that this
610 effect was most prominent in those cells that showed an increase in GABA-induced lick
611 coherence. Moreover, the information conveyed about NaCl, KCl and NH_4Cl was increased by
612 GABA enhancement only in those cells where GABA also increased lick coherence. The largest
613 effect of increasing GABAergic tone was seen in the discrimination of palatable (sucrose and
614 NaCl) vs. unpalatable (citric acid and quinine) tastants. This effect was apparent regardless of the
615 effect of GABA on lick coherence – though again, more prominent in cells in which GABA

616 enhanced lick coherence. Interestingly, this change was only manifested at longer taste response
617 intervals (1-2 s), which is considered to be the critical period for judging palatability in the
618 gustatory cortex (Katz et al. 2001); our finding of a similar time-dependence in the brainstem
619 suggests that the action of GABA in the brainstem may be involved in this effect.
620 Lick coherence is common in the brainstem taste areas of awake unrestrained animals
621 (Denman et al. 2019; Roussin et al. 2012; Weiss et al. 2014); most NTS cells, including most
622 taste-responsive cells, show some degree of lick coherence. We have found that, in addition to
623 taste-responsive cells, non-taste-responsive cells that show significant lick coherence can also
624 convey some information about taste quality (Denman et al., 2019; Roussin et al., 2012; Weiss et
625 al., 2014). Thus, the lick pattern, as reflected in the lick coherent spiking of these cells, can
626 buttress the information about taste quality conveyed by taste-evoked activity. GABA
627 enhancement in NTS was found to alter lick coherence during taste stimulus presentation only in
628 taste-responsive cells. Thus, our data suggest that GABAergic activity may modulate taste-
629 related lick coherence to amplify the contributions of some cells while diminishing the
630 contributions of others to the neural representation of taste in the rNTS. Collectively, these
631 effects essentially reconfigure the sensorimotor balance among taste-responsive neurons in
632 rNTS.
633 The effects of GABA activation reported here must be considered in the context of some
634 obvious limitations. For example, the amplification of GABAergic tone via optogenetic
635 stimulation is a non-physiological manipulation. Under normal physiological conditions, cells in
636 the rNTS are under a tonic inhibitory influence, with GABA as a major contributor (Grabauskas
637 and Bradley, 2003; Liu et al., 1993; Smith and Li, 1998). Moreover, taste simulation may evoke
638 GABA release in rNTS. Experimental augmentation of GABA release during taste stimulation

639 represents at best a crude exaggeration of the natural influence of GABA on rNTS cells.
640 Nevertheless, the fact that there were consistent effects on taste responsivity within individual
641 cells and systematic effects on a population level implies that there are meaningful concepts that
642 can be derived from our results.
643 The fact that global optogenetic activation of GABA activates GABA release from a variety
644 of sources represents another limitation of the present study and could be in part responsible for
645 the diversity of effects seen. GABAergic projections arise from both local interneurons in NTS
646 (Davis 1993; Lasiter and Kachele, 1988) as well as centrifugal structures such as the gustatory
647 cortex (GC; Smith and Li, 2000; Torrealba and Muller, 1996) or amygdala (AMG; Batten et al.,
648 2002; Saha et al., 2002). Further, stimulation of the solitary tract can monosynaptically activate
649 GABAergic NTS cells (Boxwell et al., 2013), suggesting that afferent input can initiate
650 feedforward inhibition. Although input from the gustatory cortex is mainly glutaminergic
651 (Torrealba and Muller, 1996), some cortical input to the NTS makes connections to GABAergic
652 interneurons (Smith and Li ,2000). Temporary pharmacological elimination of GC input to NTS
653 shows similar effects to that reported here: responses to some tastants were attenuated while
654 others were enhanced, sometimes within the same cell (Di Lorenzo and Monroe, 1995). These
655 data suggest that the effects of GABA activation may be at least partially accounted for by
656 mimicking GC input to NTS. Another potential source of GABAergic influence may be the
657 AMG. While anatomical evidence suggests that AMG input to the NTS is inhibitory (Batten et
658 al., 2002; Saha et al., 2002), physiological studies suggest that the effect of stimulation of AMG-
659 NTS input is excitatory (Cho et al., 2003), suggesting the possibility that the AMG generates a
660 disinhibitory effect in the NTS (see Herman et al., 2012). If true, that might contribute to the
661 enhanced responses that became apparent following enhanced GABA release. Since the AMG

662 supplies a rich centrifugal innervation to the rostral NTS (Kang and Lundy, 2009), GABA-
663 induced enhancement of information about palatable vs. unpalatable tastants might be mainly
664 due to GABA release from AMG-NTS projections.
665
666 ***Conclusions***
667 The effects of GABA release during taste stimulation was studied in the NTS of awake,
668 unrestrained rats. GABA changed the taste response profile in about half of the taste responsive
669 cells that were recorded, but the overall interrelationships among the taste-evoked across unit
670 patterns were not altered. Interestingly, GABA activation did not result in more narrow tuning in
671 taste-responsive cells as might have been predicted from studies conducted in anesthetized
672 animals (Smith and Li, 1998). Instead, the population response was essentially remodeled by
673 shifting the identities of the cells conveying specific stimulus-related signals. The coherence of
674 spike activity with the lick pattern was also altered by GABA activation but primarily in taste-
675 responsive cells. In those cells where GABA activation enhanced taste-related lick coherence,
676 information conveyed by temporal coding about taste quality was increased. Most notably, taste-
677 driven GABA activation increased the information conveyed by the temporal characteristics of
678 taste responses about palatability, especially in neurons with GABA-induced shifts in lick
679 coherence. In all, this study shows that GABAergic activation remodels the global population
680 response to taste by both shifts in the responses to taste and the extent to which neural activity
681 reflects licking. Future experiments should tease apart the effects of the various sources of
682 GABAergic activity to obtain a more precise picture of the role of GABA in the rostral NTS.
683
684

685 **REFERENCES**

686

687 Batten TF, Gamboa-Esteves FO, Saha S (2002) Evidence for peptide co-transmission in
688 retrograde- and anterograde-labelled central nucleus of amygdala neurones projecting to NTS.
689 Auton Neurosci 98:28-32.

690

691 Benjamini Y, Hochberg Y. (1995) Controlling the false discovery rate: a practical and powerful
692 approach to multiple testing. J Royal Stat Soc, Series B 57(1):289–300.

693

694 Boxwell AJ, Yanagawa Y, Travers SP, Travers JB (2013) The mu-opioid receptor agonist
695 DAMGO presynaptically suppresses solitary tract-evoked input to neurons in the rostral solitary
696 nucleus. *J Neurophysiol* 109:2815–2826.

697

698 Breza JM, Nikonov AA, Contreras RJ (2010) Response latency to lingual taste stimulation
699 distinguishes neuron types within the geniculate ganglion. J Neurophysiol 103:1771–1784.

700

701 Cho YK, Li CS, Smith DV (2002) Taste responses of neurons of the hamster solitary nucleus are
702 enhanced by lateral hypothalamic stimulation. J Neurophysiol 87(4):1981-92.

703

704 Cho YK, Li CS, Smith DV (2003) Descending influences from the lateral hypothalamus
705 and amygdala converge onto medullary taste neurons. Chem Senses 28:155–171.

706

707 Davis BJ (1993) GABA-like immunoreactivity in the gustatory zone of the nucleus of the
708 solitary tract in the hamster: light and electron microscopic studies. Brain Res Bull 30(1-2):69-
709 77.
710
711 Denman AJ, Sammons JD, Victor JD, Di Lorenzo PM (2019) Heterogeneity of neuronal
712 responses in the nucleus of the solitary tract suggests sensorimotor integration in the neural code
713 for taste. J Neurophysiol 121(2):634-645.
714
715 Di Lorenzo PM, Lemon, CH (2000) The neural code for taste in the nucleus of the solitary tract of the
716 rat: Effects of adaptation. Brain Res 852: 383-97.
717
718 Di Lorenzo PM, Lemon CH, Reich CG (2003) Dynamic coding of taste stimuli in the brain stem:
719 effects of brief pulses of taste stimuli on subsequent taste responses. J Neurosci 23:8893-8902.
720
721 Di Lorenzo PM, Monroe S (1995) Corticofugal influence on taste responses in the nucleus of the
722 solitary tract in the rat. *J Neurophysiol* 74(1):258-272.
723
724 Escanilla OD, Victor JD, Di Lorenzo PM (2015) Odor-taste convergence in the nucleus of the
725 solitary tract of awake freely licking rats. J Neurosci 35(16):6284-6297.
726
727 Gompf HF, Budygin EA, Fuller PM, Bass CE (2015) Targeted genetic manipulations of neuronal
728 subtypes using promoter-specific combinatorial AAVs in wild-type animals. Front Behav
729 Neurosci 9:152.

730

731 Grabauskas G, Bradley RM (1998) Tetanic stimulation induces short-term potentiation of
732 inhibitory synaptic activity in the rostral nucleus of the solitary tract. *J Neurophysiol* 79(2):595-
733 604.

734

735 Grabauskas G, Bradley RM (1999) Potentiation of GABAergic synaptic transmission in the
736 rostral nucleus of the solitary tract. Neurosci 94(4):1173-82.

737

738 Grabauskas G, Bradley RM (2003) Frequency-dependent properties of inhibitory synapses in the
739 rostral nucleus of the solitary tract. J Neurophysiol 89:199-211.

740

741 Herman M A, Gillis RA, Vicini S, Dretchen KL, Sahibzada N (2012) Tonic GABAA
742 receptor conductance in medial subnucleus of the tractus solitarius neurons is inhibited
743 by activation of mu-opioid receptors. J Neurophysiol 107:1022-1031.

744

745 Hirata S-i, Nakamura T, Ifuku H, Ogawa H (2005) Gustatory coding in the precentral extension
746 of area 3 in Japanese macaque monkeys; comparison with area G. Exp Brain Res 165:435–446.

747

748 Kattla S, Lowery MM (2010) Fatigue related changes in electromyographic coherence between
749 synergistic hand muscles. Exp Brain Res. 202(1):89-99.

750

751 Kang Y, Lundy RF (2009) Terminal field specificity of forebrain efferent axons to brainstem
752 gustatory nuclei. Brain Res 1248:76-85.

753

754 Katz DB, Simon SA, Nicolelis MA(2001) Dynamic and multimodal responses of gustatory
755 cortical neurons in awake rats. J Neurosci 21:4478–4489.

756

757 Lasiter PS, Kachele DL (1988) Organization of GABA and GABA-transaminase containing
758 neurons in the gustatory zone of the nucleus of the solitary tract. Brain Res Bull 21:623-636.

759

760 Leonard NL, Renehan WE, Schweitzer L (1999) Structure and function of gustatory neurons in
761 the nucleus of the solitary tract. IV. The morphology and synaptology of GABA-immunoreactive
762 terminals. Neurosci 92(1):151-62.

763

764 Li CS, Cho YK, Smith DV (2002) Taste responses of neurons in the hamster solitary nucleus are
765 modulated by the central nucleus of the amygdala. J Neurophysiol 88:2979–2992.

766

767 Liu H, Behbehani M, Smith DV (1993)The influence of GABA on cells in the gustatory region
768 of the solitary nucleus. Chem Senses 18:85-305.

769

770 Matsuo R, Shimizu N, Kusano K (1984) Lateral hypothalamic modulation of oral sensory
771 afferent activity in nucleus tractus solitarius neurons of rats. J Neurosci 4:1201–1207.

772

773 Monroe S, Di Lorenzo PM. (1995) Taste responses in neurons in the nucleus of the solitary tract
774 that do and do not project to the parabrachial pons. J Neurophysiol 74(1):249-57.

775

776 Mukherjee N, Wachutka J, Katz DB (2017) Python meets systems neuroscience: affordable,
777 scalable and open-source electrophysiology in awake, behaving rodents. Proc. of the 16th Python
778 in Science Conf. SciPy.
779
780 Murzi E, Hernandez L, Baptista T (1986) Lateral hypothalamic sites eliciting eating affect
781 medullary taste neurons in rats. Physiol Behav 36:829–834.
782
783 Panzeri S, Senatore R, Montemurro MA, Petersen RS (2007) Correcting for the sampling bias
784 problem in spike train information measures. J Neurophysiol 98:1064–1072.
785
786 Rainer G, Asaad WF, Miller EK (1998) Selective representation of relevant information by
787 neurons in the primate prefrontal cortex. Nature 393:577-9.
788
789 Rosen AM, Di Lorenzo PM (2009) Two types of inhibitory influences target different groups of taste-
790 responsive cells in the nucleus of the solitary tract of the rat. Brain Res 1275:24-32.
791
792 Roussin AT, D'Agostino AE, Fooden AM, Victor JD, Di Lorenzo PM (2012) Taste coding in the
793 nucleus of the solitary tract of the awake, freely licking rat. J Neurosci 32(31):10494-506.
794
795 Saha S, Henderson Z, Batten TF (2002) Somatostatin immunoreactivity in axon terminals in rat
796 nucleus tractus solitarii arising from central nucleus of amygdala: coexistence with GABA and
797 postsynaptic expression of sst2A receptor. J Chem Neuroanat 24:1-13.
798

799 Sammons JD, Weiss MS, Escanilla OD, Fooden AF, Victor JD, Di Lorenzo PM (2016) Spontaneous
800 changes in taste sensitivity of single units recorded over consecutive days in the brainstem of the awake
801 rat. PLoS One 11(8):e0160143.
802
803 Smith DV, Li C-S (1998) Tonic GABAergic inhibition of taste-responsive neurons in the nucleus
804 of the solitary tract. Chem Senses 23:159-169.
805
806 Smith DV, Li C-S, Davis BJ. (1998) Excitatory and inhibitory modulation of taste responses in
807 the hamster brainstem. Ann N Y Acad Sci. 855:450-6.
808
809 Smith DV, Li C-S (2000) GABA-mediated corticofugal inhibition of taste-responsive neurons in
810 the nucleus of the solitary tract. Brain Res 858:408–415.
811
812 Smith DV, Travers JB (1979) A metric for the breadth of tuning of gustatory neurons. Chem
813 Senses 4(3):215-29.
814
815 Torrealba F, Muller C (1996) Glutamate immunoreactivity of insular cortex afferents to the
816 nucleus tractus solitarius in the rat: a quantitative electron microscopic study. Neurosci 71:77-87.
817
818 Wakabayashi KT, Feja M, Baindur AN, Bruno MJ, Bhimani RV, Park J, Hausknecht K, Shen R-
819 Y, Haj-Dahmane S, Bass CE (2019) Chemogenetic Activation of Ventral Tegmental Area
820 GABA Neurons, but Not Mesoaccumbal GABA Terminals, Disrupts Responding to Reward-
821 Predictive Cues. Neuropsychopharm 44(2):372-380.

822

823 Weiss MS, Victor JD, Di Lorenzo PM (2014) Taste coding in the parabrachial nucleus of the
824 pons in awake, freely licking rats and comparison with the nucleus of the solitary tract. J
825 Neurophysiol 111:1655–1670.

826

827 Xiao X, Li J, Samulski RJ (1998) Production of high-titer recombinant adeno-associated virus
828 vectors in the absence of helper adenovirus. J Virol 72:2224–2232.

829

830 Yizhar O, Fenno LE, Davidson TJ, Mogri M, Deisseroth K. (2011) Optogenetics in neural
831 systems. Neuron 71(1): 9-34.

832

FIGURE LEGENDS

Fig. 1. A. Schematic diagram of the experimental protocol. Each vertical line represents a lick. Colored lines represent reinforced licks; black lines represent dry licks. Taste stimuli were presented as five consecutive reinforced licks. A random half of the taste stimuli were accompanied by laser activation that lasted 1 s following each tastant lick. Between taste stimulus trials, six AS licks were presented on a VR5 schedule. Note that AS was used both as one of the taste stimuli and as the rinse for all of the tastants. B. Thirty sec sequence of licks from an actual test session showing taste stimulus presentations with and without laser. Note that in Panel A, the horizontal axis is schematic and shows the licks equally-spaced; in Panel B, the horizontal axis is time, and the tickmarks indicate actual lick timing.

Fig. 2. Examples of the effects of GABA activation on taste stimuli in six different cells. In both A. and B., top row shows responses without GABA stimulation; bottom row shows responses with GABA enhancement. Top of each panel shows raster plots of each trial with black lines/dots signifying the occurrence of a spike. Colored triangles indicate reinforced licks. Light blue triangles show AS licks. Bottom of each panel shows a PSTH of the response, in spikes per second. Shaded area indicates the presence of the laser. For lick-by-lick responses, the laser is presented for the entire lick-by-lick response. A. Examples of five-lick responses with and without GABA stimulation in three different cells. B. Examples of lick-by-lick responses from three different cells with and without GABA stimulation. C. Examples of the effects of laser application in the rNTS in animals that were not infused with virus.

855 **Fig. 3.** Taste response magnitudes of rNTS neurons with and without GABA stimulation. The
856 absolute value of each response was calculated for the 27 5-lick and 41 lick-by-lick taste
857 neurons. Filled bars/diamonds indicate excitatory responses; empty bars/diamonds indicate
858 inhibitory responses. Thirteen neurons responded on both time scales. Neurons were separated
859 by their best-stimulus response without GABA stimulation (grey bars). Responses during GABA
860 stimulation (diamonds) are overlaid on the non-stimulated responses. Neurons that showed taste
861 responses only during GABA stimulation are shown at the far right.
862
863 **Fig. 4**. Pie graphs showing the distribution of the number of responses to each of the five basic
864 tastants (top) and the number of tastants to which a neuron responds (bottom). Graphs on the left
865 are distributions without GAB activation; graphs on the right show the distributions with GABA
866 activation. Although each tastant evoked about the same number of responses across the sample,
867 neurons were significantly more narrowly tuned with GABA stimulation. See text for details.
868
869 **Fig. 5.** Multidimensional scaling of taste response magnitudes. Pearson's correlations were used
870 as a measure of similarity for across-unit patterns evoked by tastants. A dashed line connects the
871 patterns evoked by taste stimuli without GABA stimulation; a solid line connects the patterns
872 evoked by taste stimuli with GABA stimulation. Gutman stress values were: 1 dimension, 0.281;
873 2 dimensions, 0.108; 3 dimensions, 0.062; 4 dimensions, 0.026; 5 dimensions, 0.017. GABA
874 activation shifted the location of all taste-evoked across unit patterns, but the overall organization
875 was unchanged.
876

877 **Fig. 6.** Lick coherence in taste-responsive and non-taste-responsive neurons. **A.** Distribution of
878 overall lick coherence values for both taste (filled bars) and non-taste (hollow bars) neurons.
879 Overall lick coherence values were calculated using all licks whether the lick was reinforced or
880 not. **B.** Change in tastant-restricted lick coherence values with GABA stimulation (ordinate) with
881 taste-restricted lick coherence without GABA activation shown on the abscissa.
882
883 **Fig. 7.** Information (in bits) conveyed about taste quality in taste and non-taste neurons in rNTS.
884 For each cell, H_{max} was plotted without vs. with laser-stimulated GABA release. **A.** taste cells or
885 **B**. non-taste cells. Only neurons with at least 6 trials for each tastant were used. Information was
886 conveyed by the temporal aspects of taste responses in 33 of 54 (61%) taste cells and 18 of 59
887 (31%) of non-taste cells. GABA stimulation either enhanced or attenuated information conveyed
888 about taste quality in 73% (24 of 33) taste cells and 78% (4 of 18) non-taste cells.
889
890 **Fig. 8.** Information (in bits)conveyed about the five prototypical tastants from the population of
891 rNTS neurons over the first 2s of response. Separate analyses were conducted at each response
892 interval. Temporal coding information obtained with (solid line) and without (dashed line)
893 GABA stimulation is shown. **Left.** Information for all taste cells with 5-lick responses. **Right.**
894 Neurons were separated into groups depending on how GABA stimulation affected stimulus-
895 restricted lick coherence. Also shown is the effect of GABA stimulation on the taste-related
896 information conveyed by the lick pattern. Numbers adjacent to each data point denote number of
897 neurons in which significant taste quality information was obtained. GABA stimulation
898 enhanced the information conveyed about salty tastes only in those cells where GABA also

enhanced lick coherence. GABA stimulation did not affect the information conveyed by the lick pattern.

Fig. 9. Information (in bits) conveyed about salty tastants (NaCl, KCl, and NH_4Cl) from the population of rNTS neurons over the first 2s of response. Separate analyses were conducted at each response interval. Temporal coding information obtained with (solid line) and without (dashed line) GABA stimulation is shown. **Left.** Information for all taste ells with 5-lick responses. **Right.** Neurons were separated into groups depending on how GABA stimulation affected stimulus-restricted lick coherence. Also shown is the effect of GABA stimulation on the taste-related information conveyed by the lick pattern. Numbers adjacent to each data point denote number of neurons in which significant taste quality information was obtained. GABA stimulation enhanced the information conveyed about salty tastes only in those cells where GABA also enhanced lick coherence. GABA stimulation did not affect the information conveyed by the lick pattern.

Fig. 10. Information (in bits) conveyed about palatable (sucrose, NaCl) vs. unpalatable (citric acid, quinine) from the population of rNTS neurons over the first 2s of response. Separate analyses were conducted at each response interval. Temporal coding information obtained with (solid line) and without (dashed line) GABA stimulation is shown. **Left.** Information for all taste ells with 5-lick responses. **Right.** Neurons were separated into groups depending on how GABA stimulation affected stimulus-restricted lick coherence. Also shown is the effect of GABA stimulation on the taste-related information conveyed by the lick pattern. Numbers adjacent to each data point denote number of neurons in which significant taste quality information was

obtained. Regardless of the effect of GABA stimulation on lick coherence, GABA stimulation enhanced the information conveyed about taste palatability in taste responses >1s. GABA stimulation did not affect the information conveyed by the lick pattern.

Fig. 11. Histological reconstruction of neuronal recordings and channelrhodopsin expression in the rNTS. **A**. Schematic diagram of the brainstem with a dashed oval outlining the rNTS and the center of the lesion associated with each of the five rats from which data were collected represented by an *. Lesions ranged from 11.76-12.48 mm posterior to bregma. **B.** Image of the rNTS (dashed yellow oval); red box represents magnified inset. White scale bar represents 500µm. **C.** Magnified image of rNTS. Channelrhodopsin: green; DAPI: blue. White scale bar represents 100µm.

Table 1. Analyses of lick behavior.

Stimulus	Trials	ILI (s)	No. pauses	Pause length (s)	5 licks (s)
Sucrose	200	0.155	2	1.891	0.644
Sucrose+GABA	203	0.155	2	2.160	0.642
NaCl	194	0.150	2	1.278	0.625
NaCl+GABA	204	0.151	1	1.091	0.627
Citric acid	208	0.155	14	1.826	0.653
Citric acid+GABA	202	0.156	14	1.923	0.655
Quinine	211	0.158	30	9.005	0.668
Quinine+GABA	205	0.157	27	1.622	0.657
MSG	204	0.147	3	1.730	0.628
MSG+GABA	201	0.148	0	NA	0.622
KCl	206	0.153	13	1.483	0.627
KCl+GABA	200	0.155	9	1.281	0.646
NH_4Cl	199	0.147	11	1.227	0.616
NH_4Cl+GABA	202	0.148	8	2.156	0.623
AS	207	0.161	19	1.663	0.667
AS+GABA	201	0.161	9	3.729	0.680

Trials, total number of trials for each stimulus across animals; ILI, median interlick interval; No. of pauses, the median number of pauses within a 5-lick taste stimulus trial; Pause length, the median duration (s) of pauses that occurred within a 5-lick taste stimulus trial; 5 licks, the median time (s) to complete all five licks of a stimulus trial.

Table 2. Effect of GABA stimulation on taste response magnitudes in rNTS.

5-Lick resp.*	Sucrose	NaCl	MSG	Citric acid	Quinine	KCl	NH_4Cl	Art. Saliva
Increased	0	1	1	1	3	1	1	0
Decreased	2	1	0	1	0	1	1	2
No change	10	15	12	12	9	8	14	4
Lick-by-lick**								
Increased	3	2	2	0	2	0	2	4
Decreased	0	3	2	1	3	0	1	2
No Change	20	20	21	19	15	11	22	20
All responses***								
Increased	2	2	3	1	5	1	4	3
Decreased	2	4	2	1	3	1	1	4
No Change	26	29	28	29	22	16	28	23

*10 of 27 (37%) cells affected
**16 of 41 (39%) cells affected
***22 of 54 (41%) cells affected