Title: Neural Coding of Gustatory Information in the Nucleus of the Solitary Tract in Lean and Obese Awake Freely-licking Rats

Abbreviated Title: Taste coding in the NTS of obese and lean rats

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Abstract

Taste perception changes with obesity but the underlying neural changes remain poorly understood. To address this issue, we recorded taste responses from single cells in the nucleus tractus solitarius (NTS, the first central synapse in the gustatory circuit) in awake, diet-induced obese [(DIO; ≥ 8 wks on a high-energy diet (HED))] and lean rats. Rats were implanted with a bundle of microelectrodes in the NTS and allowed to recover. Water-deprived rats were allowed to freely lick various tastants in an experimental chamber. Taste stimuli included an array of sapid stimuli dissolved in artificial saliva (AS). Each taste trial consisted of 5 consecutive licks followed by 5 AS licks presented on a VR5 schedule. Results showed that taste responses in NTS cells in DIO rats ($n=49$) were smaller in magnitude, shorter in duration and longer in latency than those in lean rats ($n=74$). However, there were proportionately more taste-responsive cells in DIO than in lean rats. Lick coherence in DIO rats was significantly lower than in lean rats, both in taste-responsive and lick-related cells ($n=172$ in lean; $n=65$ in DIO). Analyses of temporal coding showed that taste cells in DIO rats conveyed less information about taste quality than cells in lean rats. Collectively, results suggest that a HED produces blunted, but more prevalent, responses to taste in the NTS and a weakened association of taste responses with ingestive behavior. These neural adaptations may represent both negative effects and compensatory mechanisms of a HED that may underlie deficits in taste-related behavior associated with obesity.

Significance Statement

The state of obesity, the prevalence of which is a growing epidemic worldwide, is accompanied by changes in taste-related behavior. Here we report that neural responses to taste stimuli in the nucleus tractus solitarius (NTS) of obese rats are blunted and convey less information about taste quality than those in lean rats. These effects are perhaps offset by several compensatory mechanisms, suggesting that the neural representation of taste stimuli in the NTS may be remodeled to adjust to the metabolic state induced by a high fat diet.

Introduction

The idea that obesity, an epidemic for U.S. adults (Ogden et al. 2015), is caused by a chronic surplus of energy from food belies the complexity of its etiology and the obesity-related changes that occur in the body. Along with a constellation of hormonal and physiological changes that accompany obesity, alterations in the taste system are also evident. For example, an increase in visceral fat is negatively correlated with both olfactory and taste function (Fernandez-Garcia 2017). In both humans and rodents, taste sensitivity changes with body weight, specifically for taste qualities that signal high-energy availability, e.g. sweet and fatty (Bartoshuk et al. 2006; Hajnal et al. 2005; Shin et al. 2011; Zhang et al. 2011, 2013; Duca et al. 2014; Dando 2015).

The gustatory system serves as a feed-forward regulator of the ingestive neural circuitry. Taste serves as a sensory gate-keeper, eliciting different sensations depending on a food's chemical composition (Scott and Mark 1987; Spector and Travers 2005; Chandrashekar et al. 2006) and acting as a mediator of food consumption (Berthoud 2002; Zocchi et al. 2017). Thus, it is unsurprising that there are multiple taste-related neural structures that are sensitive to an animal's internal state. For example, a high protein diet (Darcel et al. 2005), changes in blood glucose (Giza and Scott 1983), changes in blood insulin (Giza and Scott 1987), gastric distention (Baird et al. 2001) and obesity (Kovacs and Hajnal 2008) can all alter taste processing in the brain.

The nucleus tractus solitarius (NTS), the first synapse in the mammalian central gustatory pathway, is a point of convergence for taste processing and signals that inform the organism of its internal state. NTS neurons express a wide array of receptors involved in the homeostatic regulation of ingestion (Ritter 2000; Faulconbridge et al. 2003; Rozengurt 2006; Cui et al. 2011;

Blouet and Schwartz 2012; Zhan et al. 2013). Moreover, there is a large degree of intranuclear connectivity bridging the caudal viscerosensory pole with the rostral orosensory pole (Beckman and Whitehead 1991; Ganchrow et al. 2014; Travers et al. 2018). With reciprocal connectivity from forebrain structures controlling feeding behavior such as the amygdala and hypothalamus (Whitehead et al. 2000) along with parallels in the vagal-visceral and lingual pathways (Karimnamazi et al. 2002), the gustatory system is a highly interactive and integrative system with the NTS being a crucial first node in the feeding circuit.

Here, we aimed to detail the effects of obesity on responses to taste stimuli in the NTS. We recorded taste responses from single cells in the NTS of awake, freely licking rats that had been made obese by exposure to a high energy diet (HED), i.e. diet-induced obesity (DIO). We then compared cellular responses to taste and licking in DIO rats to those in lean rats maintained on standard lab chow. Results showed that taste responses in DIO rats are blunted compared with those in lean rats and their taste-evoked spike trains convey less information about taste quality. In addition, the relative proportion of taste cells was increased in the NTS of DIO rats, suggesting a compensatory mechanism for diminished taste responsivity in DIO rats.

Materials and Methods

Subjects

Male Sprague-Dawley rats (N = 78) were obtained from Taconic Labs, Inc. (Germantown, NY) and allowed to acclimate to the vivarium for a minimum of seven days with a 12 hr light/dark cycle (lights off at 0900hr). Animals were then randomly divided into two groups, where one group (diet-induced obese, DIO; n = 39) was fed a high-fat, high sugar diet (high energy diet, HED; 45% kCal from fat, 17% sucrose, Research Diets D12451, New Brunswick, NJ) starting at 8 wks of age for a minimum of 8 wks before surgical implantation and throughout testing until they were sacrificed. The lean control group (n = 39) was fed a standard chow diet (Purina Lab Diet 5001, 13.5% kCal from fat, 3.7% sucrose, Lab Diet, St. Louis, MO) for the duration of the experiment.

Body Composition Monitoring

Before being placed on a HED, and after 8 wks on a HED, DIO animals underwent a Dual-energy X-ray Absorptiometry (DXA) scan to determine body composition. Animals were sedated with Medetomidine HCl (Pfizer Inc., NY, USA; 0.1 mg/kg, s.c.) and placed on a scanning bed where body composition was calculated by Hologic APEX Discovery A software (Hologic, Bedford, MA). Scan results determined both body fat and lean tissue mass. Sedation was reversed by Atipamezole (Pfizer Inc., NY, USA; 0.1 mg/kg, i.p.) upon completion of the scan.

Taste Stimuli and Delivery System

Taste stimuli included both traditional and naturalistic stimuli (Sammons et al. 2016). Traditional taste stimuli included: 0.05M NaCl (N), 0.1 M and 0.5 M sucrose (Lo Su and Hi Su,

respectively), 0.01 M citric acid (A), and 0.002 M caffeine (Caff). These solutions were made from reagent grade chemicals purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Pittsburg, PA). Naturalistic taste stimuli included clam juice (Clm J; containing 0.12 M NaCl), 25% and 100% grape juice (Lo GJ and Hi GJ respectively; containing 0.12 M and 0.48 M sucrose), lemon juice (Lm J; containing 0.01 M citric acid), coffee (Coff; containing 0.002 M caffeine) and 25% heavy cream (to serve as a fat taste stimulus). All stimuli were dissolved in artificial saliva (AS; 0.015 M NaCl, 0.022 M KCl, 0.003 M CaCl₂; 0.0006 M MgCl₂; pH ~ 7.4; Hirata et al. 2005; Breza et al. 2010).

Electrophysiological testing was conducted in an operant chamber (MED Associates, St. Albans, VT), where rats could navigate freely. Pressurized (~11 psi) 35 mL tubes mounted on the exterior of the testing chamber served as reservoirs for tastant solutions. Tastant reservoirs were connected to 20-gauge stainless steel tubes housed within a larger stainless steel tube (8mm dia.) to form a single lick spout. Delivery of tastant solutions was controlled by solenoids (Parker-Hannifin, Fairfield, NY) triggered by licks. Stimulus trials were controlled by MED Associates software (MED Associates, St. Albans, VT). Lick-evoked fluid delivery was individually calibrated daily for each tastant reservoir so that $12 \pm 1 \mu\text{l}$ of fluid were delivered immediately after the animals' tongue broke an infrared beam located in front of the lick spout.

Stimulus delivery for electrophysiological experiments was as follows: a stimulus trail consisted of a block of five consecutive taste stimulus licks, separated by five “rinse” (AS) licks, each of which was presented on a variable ratio 5 schedule. That is, each AS lick was separated by four to six “dry” (non-reinforced) licks, except when AS serves as a “taste” stimulus. Taste stimulus trials were presented in a pseudorandomized order; to encourage the animal to lick the tastant delivery spout, Lo Su (0.1M sucrose) was the first stimulus encountered by the animal in each session.

Microwire Implantation Surgery

To implant the microwire assembly, animals underwent a surgical implantation procedure, performed under aseptic conditions. Animals were given 0.05 mg/kg Buprenorphine HCl and 0.05 mg/kg Atropine Sulfate (VETone, Boise, ID) subcutaneously approximately 1 hr before being anesthetized. Anesthesia was induced with 4% isoflurane and the dosage was tapered down throughout the surgery (1-3% maintenance). Vital signs (e.g. blood oxygenation, heart rate, respiration rate and body temperature) as well as reflexive behaviors (e.g. toe pinch reflex) were monitored throughout the surgery to ensure a surgical plane of anesthesia. Body temperature was maintained at 37° C by an autoregulating heating pad connected to a rectal thermistor. Artificial tear gel (AltaLube, Altaire Pharmaceuticals, Aquebogue, NY) was applied to the eyes to prevent drying. The crown of the head was shaved and the animal was then placed in the stereotaxic apparatus (Kopf Model 1900, Tujunga, CA).

Once the animal was in the stereotaxic instrument, the head was swabbed with Betadine / iodine scrub followed by a 70% ethanol solution three times and the animal was given an injection of 5 mL sterile lactated Ringers saline solution s.c. (Hospira, Lake Forest, IL). An incision was then made along the midline and the underlying fascia was gently retracted. Six pilot holes were then drilled for implantation of bone screws, which were used as anchors for the dental acrylic head-cap. Additionally, one skull screw served as an electrical ground for the microwire electrode array. The head was then angled so that bregma was 25° below lambda. A small hole (~2mm dia.) was drilled in the occipital bone (~ 14 – 15.3 mm posterior to bregma and ~1.5-2.5 mm lateral to the midline) and the underlying dura was punctured and resected for the insertion of the microwire electrode assembly (Innovative Neurophysiology, Durham, NC, USA). For implantation of the chronic microdrive, the probe was implanted at ~4.5 mm ventral

to the cerebellar surface, and the shuttle mechanism on the probe was used to advance the wires to the target post-operatively. The hole was sealed with a biocompatible silicone elastomer (Kwik-Cast, WPI Instruments, Sarasota, FL, USA) and the electrode was secured with an acrylic resin. Once the acrylic cured and the electrode was secured to the head, the animal received a 10 mL injection of warmed saline and was given 100% oxygen @ 1.5 L/min until the animal was spontaneously mobile. Once the animal was ambulatory, Buprenorphine HCl (0.05 mg/kg, s.c.) and Gentamicin (6 mg/kg, s.c.) were given as an analgesic and antibiotic, respectively, for three days. Topical antibiotic with pain reliever (Neosporin) was applied around the head-cap daily for five days to prevent infection. Animals in post-operative care were given sterile isotonic saline or a lactated ringer's solution (10-15 mL s.c.) as needed to prevent dehydration. Animals were kept in post-operative recovery for a minimum of seven days and until they regained their pre-operative weight.

Electrophysiological Recordings

Prior to testing, animals were water deprived for 22 hrs. Animals were placed in an operant chamber and a head-stage and cable were attached to the animals' electrode assembly. A house light inside the operant chamber signaled the beginning of a recording session. Neural activity (timestamps of single waveforms, with 25 μ s resolution) as well as the identity of stimulus deliveries were obtained using Sort Client software (Plexon, Dallas, TX). After recording sessions, waveforms were exported to Offline Sorter (Plexon, Dallas, TX) for individual unit isolation. Criterion for isolation was a refractory period of > 2 ms and distinct clusters in principal component feature space (Stapleton et al. 2006). After recording sessions, animals were returned to their home cages. After 1 hr in their home cages, rats were given 1 hr of water access. Each animal experienced repeated daily (except for weekends) recording sessions

until there were no more isolatable units and/or until the electrode assembly was past the NTS.

Data Analysis - General

Spontaneous firing rate was determined by obtaining the mean and standard deviation (SD) of firing rate (in spikes/sec; sps) from multiple 14 s samples during which there was no licking. The first 3 s of the 14 s-window served to ensure that there was no stimulus left in the mouth to exclude any potential stimulus-evoked firing. The next 10 s is the period where spontaneous firing rates were calculated. The 1 s-window after the spontaneous period was calculated served to ensure there was no firing rate change due to anticipatory behavior of licking. Baseline firing rate for a given taste stimulus was calculated as the average firing rate (in sps) during the 1s of activity before the first stimulus lick across all trials.

Responses were calculated for two time scales: 5-lick responses where the first lick (of five stimulus licks served as time zero and the response interval extended over 4 s, and lick-by-lick responses where each taste stimulus lick served as time zero and the response interval extended over 170 ms.

For 5-lick responses, taste stimulus-evoked activity was defined as a significant change in firing rate from baseline activity. A significant change from baseline was determined by taking a 100 ms window, moved in 20 ms increments until the firing rate was 2 SDs (95% confidence interval) above or below the baseline firing rate for three consecutive 100 ms bins. The trailing edge of the first bin that was significantly above baseline was defined as the response latency. Once this bin was identified, the 100 ms window was moved until the response was no longer significantly different from baseline. Response magnitude was determined by subtracting the taste-evoked firing rate from baseline firing rate.

For lick-by-lick responses, a significant taste response was calculated by taking a 10 ms

window, moved in 2 ms increments until the firing rate was 2 SDs above or below the baseline firing rate for three consecutive 10 ms bins. The baseline firing rate was calculated from a 50 ms pre-trial window. The trailing edge of the first bin that was significantly above baseline was defined as the response latency. Once this bin was identified, the 10 ms window was moved until the response was no longer significantly different from baseline. Response magnitude was determined by subtracting the taste-evoked firing rate from baseline firing rate.

To determine if differences existed between the proportional frequency of neurons responding on a lick-by-lick basis, 5-lick basis, or both, a Mann-Whitney U Test was applied. Other statistical tests used are described throughout the results section. Statistical calculations were done using GraphPad Prism 7.0 software. For all statistical tests, an $\alpha = 0.05$ was considered significant.

Data Analysis – Temporal Coding

To assess the contribution of the temporal characteristics of a taste-evoked spike train to taste coding, metric space analysis (MSA; Victor and Purpura 1996, 1997) was used. This analytic approach was used previously (Roussin et al. 2012; Weiss et al. 2014; Escanilla et al. 2015; Sammons et al. 2016) and is described briefly here.

MSA first quantifies the dissimilarity, or distance, between spike trains calculated as the “cost” of transforming one spike train into another by adding, removing, or moving spikes in time. The first metric is called D^{count} , where moving spikes in time has no cost; adding or deleting spikes has a cost of 1. This measure is simply the difference in the number of spikes between two taste-evoked spike trains. A second metric, $D^{spike}[q]$, takes the timing of individual spikes into account. Here, the cost of adding or deleting spikes is parameterized by q , a measure of temporal precision, such that the cost of moving a spike in time is qt ; q is in units of $1/q$ s.

To estimate the amount of information (H) conveyed by spike trains, the degree to which pairs of responses to the same stimulus differed from pairs of responses to different stimuli was determined at varying levels of temporal precision (q) by calculating the mutual information between the inferred stimulus labels and the true stimulus labels. The level of q at which information is greatest is termed H_{max} . Information when q is set to 0 is termed H_{count} .

Two analyses constructed from synthetic data sets served as controls. Shuffled analyses randomly assigned stimulus labels to taste responses and recalculated information. These analyses were performed 40 times at each level of q . The resulting information value was termed $H_{shuffled}$. Exchange analyses assessed the extent to which the rate envelope of the response is informative; spikes were reordered randomly in time while preserving the progression in time of the overall firing rate in the response. This process was repeated 10 times for each level of q . Information resulting from this analysis was termed $H_{exchange}$. If H_{max} was greater than $H_{shuffled} + 2SD$, it was concluded that the taste-evoked spike trains conveyed a significant amount of information about taste quality. The circumstance where H_{max} was greater than $H_{shuffled} + 2SD$ and equal to H_{count} implied that the taste-evoked firing rate was more informative than either the rate envelope or spike timing. If H_{max} was greater than $H_{shuffled} + 2SD$ but not greater than $H_{exchange}$, it was concluded that the rate envelope of a response conveyed a significant amount of information about taste quality. Finally, if H_{max} exceeded $H_{shuffled} + 2SD$ and $H_{exchange}$, it was concluded that spike timing contributes to the neuron's ability to distinguish among taste stimuli.

To assess the contribution of temporal and rate coding in NTS cells from DIO and lean rats, the average amount of information across the entire sample was calculated at 200, 500, 1000, 1500 and 2000 ms of response. First, the amount of information conveyed about taste quality was calculated using responses to 0.1 M sucrose, 0.05 M NaCl, 0.01 M citric acid and 0.002 M caffeine for traditional taste stimuli and 25% grape juice, 75% clam juice, lemon juice,

and coffee for naturalistic taste stimuli. At each time point, the amount of information (H_{max} , in bits) conveyed by each cell where $H_{max} > H_{shuffled} + 2SD$ was calculated. For cells that did not meet that criterion at a given time point, information was set to zero. Values of H at $q=0$ were used as an index of rate coding in cells with $H_{max} > H_{shuffled} + 2SD$. The average value of H at $q=0$ and the average value of H_{max} across the entire sample were calculated at each time point, as well as the proportion of cells with $H_{max} > H_{shuffled} + 2SD$. To determine if differences existed in the cumulative proportional frequency distribution of the neurons utilizing temporal coding (either rate envelope or spike timing) in lean and DIO rats, a Kolmogorov-Smirnov test was used. An $\alpha = 0.05$ was considered significant.

Histology

To confirm the location of an electrode, animals were deeply anesthetized (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI, USA), and direct current (1mA; 10-15s) was passed through the electrode corresponding to the channel from which a taste-responsive neuron was recorded. Animals were then perfused transcardially with isotonic saline followed by a 10% paraformaldehyde solution. Brains were removed and placed in 10% formalin for a minimum of one week. Before sectioning, brains were rinsed with a phosphate buffer saline (PBS) three times, and then left in a 20% sucrose in PBS for a minimum of 24 hrs. Brains were then frozen and sectioned at a thickness of 35 μ m through the medulla on a cryostat (Leica CM1850, Wetzlar, Germany). Tissue was thaw-mounted on slides (Colorfrost Plus, Fisher Scientific, Pittsburg, PA), stained with cresyl violet, and the lesion/recording site was identified.

Results

Body Composition data from 13 lean rats and 7 DIO rats revealed that rats fed a 45% high fat, 17% sucrose, high energy diet (HED) had a significantly different body composition compared to animals on a standard lab diet, $F(1,54) = 62.0, p < 0.0001$ (Figure 1). Rats on a HED had a higher total weight ($t(54) = 6.81, p < 0.0001, 648.9 \pm 38.9\text{g}$ vs. $425.1 \pm 24.8\text{g}$) and significantly more body fat than lean animals, ($t(54) = 3.81, p < 0.0001, 170.9\text{g} \pm 27.5\text{g}$ of fat vs. $45.7\text{g} \pm 5.3\text{g}$ fat for DIO rats and lean rats respectively). As such, rats on a HED had a significantly higher body fat percentage, ($t(54) = 6.5, p < 0.0001, 25.3 \pm 2.9\%$ vs. $10.4 \pm 0.6\%$, for DIO and lean animals, respectively) compared to animals on a standard chow diet. There were no differences in lean body mass and bone mineral content, $t(54) = 3.07, p = 0.06$, BMC; $478.0 \pm 13.6\text{g}$ vs. $379.3.8 \pm 19.9\text{g}$.

-----Figure 1-----

Electrophysiological taste responses were recorded from 74 cells in 13 lean animals and 49 cells in 8 diet-induced obese (DIO) animals. Recorded alongside these taste responsive cells were an additional 441 non-taste-responsive units in lean animals (total $N = 515$) and 179 non-taste-responsive units in DIO animals (total $N = 219$). The mean number of taste-responsive cells recorded per animal was 5.7 ± 2.2 in lean animals and 6.1 ± 1.1 in DIO animals, the median was 3 in lean animals, 5.5 in DIO animals. The range of recorded taste-responsive cells for lean animals was 1-30. The animal that provided 30 cells had 4 channels with taste-responsive activity for two consecutive days. The range of recorded taste-responsive cells for DIO animals

was 3-10. For lean rats, data from 33 taste-responsive and 149 non-taste-responsive cells were part of a dataset analyzed and published previously (Denman et al. *in press*).

Taste-responsive cells in the NTS responded to taste stimuli at two different time scales. Some responses extended over, and sometimes past the 5-lick stimulus presentation; these were called “5-Lick responses”. Other responses occurred at a short latency (<100 ms) after each taste stimulus lick and were dissipated before the next lick; these were called “Lick-by-lick” responses. Figure 2 shows an example of these two types of taste response in two different NTS cells from lean rats.

-----Figure 2-----

The average spontaneous firing rate was taken from multiple 14 s windows when the animal was not licking. The first 3 s of this window acted as a buffer to exclude any neural activity evoked by a previously presented taste stimulus; the 10 s period following the initial 3 s was used to calculate the spontaneous firing rate. The exclusion of the final 1s in the spontaneous firing rate window ensured that there was at least 1s between the time during which spontaneous activity was measured and any potential approach to the lick spout. Spontaneous firing rates are shown in Figure 3A. There was no significant difference in spontaneous firing rate between cells in lean animals (12.18 ± 1.93 spikes/sec) and neurons in DIO rats (15.4 ± 2.4 spikes/sec; $t(121) = 1.05$, $p = 0.29$).

-----Figure 3-----

Taste-responsive cells in lean animals showed larger baseline firing rates compared with those during spontaneous firing periods, but this was not true for cells in DIO rats. Unlike spontaneous firing rates calculated when the animal was not licking, baseline firing rates were calculated from a 1 s window before the first lick of a 5-lick taste stimulus trial. The baseline firing rates of taste responsive cells in DIO rats (13.7 ± 0.5 spikes/sec) were significantly lower than those in their lean counterparts (18.9 ± 0.5 spikes/sec; $t(121) = 7.054$, $p < 0.0001$) (see Figure 3B).

In addition to cells that increased their firing rate in response to one or more taste stimuli, there were several other firing patterns that we observed in both lean and DIO animals. “Lick cells” ($n = 174$ for lean; $n = 65$ for DIO) were defined as cells that fired in phase with the lick cycle; “anti-lick cells” ($n = 65$ for lean; $n = 12$ for DIO) were cells that showed a reduced firing rate during the lick-bout usually accompanied by a phasic increase in firing rate before and after a lick bout; “lick bout” cells ($n = 9$ for lean; $n = 3$ for DIO) were cells that increased their firing rate during a lick bout, but were not taste-responsive and did not fire in phase with licks; “lick / anti-lick mix cells” were cells that showed “anti-lick” properties, and also fired in phase with the lick cycle. Figure 4 shows examples of these patterns of firing. “Non-responsive cells” did not exhibit any changes in firing patterns either related to licking or taste stimulus presentation. We classified each cell according to the most prominent feature of their firing pattern (see Denman et al. in press). For example, some taste-responsive cells also showed lick-related activity but were nevertheless classified here as solely taste-responsive. The distribution of the proportion of cells in each group, shown in Figure 5A, was significantly different between lean and DIO animals ($\chi^2(5) = 14.33$, $p = 0.0136$). A greater proportion of cells in DIO animals were taste-responsive compared to the proportion of taste-responsive cells in lean rats (28% vs. 16%, respectively).

-----Figures 4 and 5-----

Figure 5B shows the distribution of cells that responded to one, two, three or four of the traditional taste stimuli (0.1 M sucrose, 0.05 M NaCl, 0.016 M citric acid, 0.002 M caffeine); both 5-lick or lick-by-lick responses were counted. In general, cells in both groups were generally broadly tuned, with only 5% of cells in lean rats and 12% of cells in DIO rats responding exclusively to a single tastant. However, cells in DIO rats were significantly more narrowly tuned across taste qualities than cells in lean rats ($\chi^2(3) = 11.18, p = 0.0108$).

Lick-related activity of taste, lick and anti-lick/lick mix cells were quantified by measuring coherence of neural firing with licks in the 4-9 Hz range. Coherence values ranged from 0-1. Differences in the distribution of coherence values were compared using a Mann-Whitney U-Test. The coherence values of neurons in lean animals were significantly different for taste cells (median = 0.334, $n = 74$ in lean, median=0.1246, $n = 49$ in DIO, $U = 972, p < 0.0001$), lick cells (median = 0.2081, $n = 172$ in lean, median=0.0596, $n = 65$ in DIO, $U = 3624, p < 0.0001$), and lick/anti-lick mix cells (median = 0.1795, $n = 43$ in lean, median = 0.3935, $n = 19$ in DIO, $U = 234, p = 0.0071$). Figure 6 shows the average coherence plotted with the distribution of coherence values for taste, lick, and lick/anti-lick mixed cells.

-----Figure 6-----

Figure 7 shows the proportion of each response type (5-lick and lick-by-lick) for lean and DIO rats. Overall, a significantly greater proportion of cells in lean animals respond to taste stimuli than in DIO rats. This reflects the observation that NTS cells in DIO rats are more narrowly tuned than those in lean rats (see Figure 5B). Proportionately more cells in lean

animals show lick-by-lick responses (Mann-Whitney U-Test $U=30$, $p = 0.0164$) and a combination of 5-lick and lick-by-lick responses (Mann-Whitney U-Test, $U=36$, $p = 0.0403$) compared with cells in DIO animals. However, when only 5-lick responses were considered, cells in lean and DIO rats were not significantly different (Mann-Whitney U-Test, $U=47$, $p > 0.05$).

-----Figure 7-----

Results show that taste responses in NTS cells in the DIO rat are smaller in magnitude (Figures 8,9), shorter in duration (Figure 10) and longer in latency (Figures 11, 12) than NTS cells in lean rats. Although there was no effect of diet on the 5-lick response magnitude ($F(1,412) = 0.066$, $p > 0.05$), lick-by-lick taste responses were significantly smaller DIO vs. lean animals ($F(1,913) = 100.4$, $p < 0.0001$) (Figure 9). In addition, both 5-lick ($F(1,513) = 13.38$, $p = 0.0003$) and lick-by-lick ($F(1,1006) = 15.08$, $p < 0.0001$) taste responses in DIO rats were significantly shorter in duration than those in lean rats (Figure 10). Similarly, latencies of both 5-lick ($F(1,510) = 20.13$, $p < 0.0001$) and lick-by lick ($F(1, 1006) = 52.29$, $p < 0.0001$) responses were longer in cells from DIO rats compared with those in lean rats (Figures 11, 12). Figure 13 shows examples of lick-by-lick taste responses from a lean and DIO animal that illustrate these effects. Note in particular the longer latencies in the taste responses in the cell from a DIO rat.

-----Figures 8-13-----

Analyses of temporal coding

Results of metric space analyses show that the temporal characteristics of taste-evoked

spike trains in DIO rats convey significantly less information about taste quality than taste-evoked spike trains in lean rats (see Figure 14). This was true for both traditional tastants and, in the longer response intervals, for the naturalistic tastants. In addition, a greater proportion of neurons in lean animals used temporal coding to convey taste-quality information compared to neurons in DIO rats for both traditional and naturalistic taste stimuli (Kolmogorov-Smirnov, $p = 0.002$). At 2 s after the first stimulus lick; this difference was especially pronounced in traditional stimuli, 85% of neurons in lean rats used temporal coding to convey taste quality information compared to only 67% of neurons in DIO rats. For both types of taste stimuli, the information derived from spike count was similar in DIO and lean rats and was less than that when the temporal characteristics of the responses were considered.

Metric space analyses revealed a significant difference between lean and DIO animals in the proportion of lick patterns that conveyed information about traditional taste stimuli (Kolmogorov-Smirnov, $p = 0.008$) (Figure 14). There was no difference between lean and DIO rats in the proportion of lick patterns that exhibited temporal coding for naturalistic stimuli (Kolmogorov-Smirnov, $p = 0.14$). This indicates that the difference between proportions of neurons using temporal coding to convey information in lean and DIO rats is not simply a reflection of the lick pattern, but a difference in taste-quality coding.

-----Figure 14-----

An additional set of analyses focused on the ability of cells in DIO and lean rats to distinguish between different concentrations of sweet tastants, specifically 0.5 M vs. 0.1 M sucrose and 25% vs. 100% grape juice. Figure 15 shows that, for different concentrations of sucrose, cells in both DIO and lean rats conveyed similar amounts of information (Mann

Whitney U test, $ps > 0.05$); however, for different concentrations of grape juice, cells in DIO rats conveyed significantly less information than cells in lean rats, especially when longer response intervals (1.5 s and 2 s) were considered (Mann Whitney U test, $U = 1210$ and 1273 respectively; $ps \leq 0.0135$). Additionally, lick cells in lean animals were able to convey more information about the naturalistic sweet stimuli compared to those in DIO animals at 2s ($U = 3123$; $p = 0.0004$), but not before ($ps > 0.1$). Importantly, these results were not a reflection in the lick pattern, as there was no difference in the information conveyed by lick pattern between lean and DIO rats (not shown).

-----Figure 15-----

Histology

The locations of taste-responsive cells were determined by passing 1 mA of current for 10 s through the wire that corresponded to the last channel where a taste-responsive cell was recorded. Lesion locations, as shown in Figure 16, spanned from 12.1-13.1 mm posterior, and between 1-2.2mm lateral to Bregma.

-----Figure 16-----

Discussion

Electrophysiological recordings from single cells in the NTS of awake DIO and lean rats show that exposure to a HED can alter the ways in which taste is encoded. Specifically, responses to taste stimuli in DIO rats were smaller in magnitude, shorter in duration and occur at longer latencies compared with those in lean rats. Not surprisingly then, taste-responsive cells in the NTS of DIO rats are more narrowly tuned and convey significantly less information about taste quality than taste-responsive cells in lean rats. An additional 620 cells were recorded alongside taste-responsive cells in the NTS of DIO and lean rats, the great majority of which showed firing patterns that closely tracked licking behavior. These lick-related cells also conveyed information about taste quality, though NTS cells in DIO rats showed less lick coherence than those in lean rats. Changes in the relative proportion of taste-responsive and other cells as a result of a HED may be a compensatory mechanism for blunted taste. In all, the present data reveal that a HED can have profound effects on how cells in the brainstem represent taste stimuli. These effects in turn may ultimately impact food choice and body weight.

The attenuation of taste responses in the NTS of DIO vs. lean rats may be the natural response to a weaker input from the periphery. Previous reports have detailed reduced taste receptor cell expression (Zhang et al. 2011, 2013; Kauffman et al. 2018) and reduced activation of taste receptor cells (Maliphol et al. 2013) with obesity. These results may contribute to the relatively narrow tuning of NTS cells in DIO vs. lean rats. This is likely the consequence of smaller response magnitudes overall; that is, weaker input may not adequately drive taste responsivity in cells from DIO rats, resulting in fewer significant taste responses and narrower tuning. Relatedly, in humans with obesity, taste-evoked potentials are shorter and weaker (Hardikar et al. 2018) compared to those in lean control subjects. In that paper, the authors

showed that taste perception did not differ across obese and lean groups, but the reward value of palatable sucrose was suppressed in obese subjects. Collectively, these data suggest that the shorter duration of taste responses in obese subjects, both rodents (present data) and humans (Haridikar et al. 2018) might underlie the lower hedonic responses to palatable taste stimuli in overweight subjects that have been reported previously (Bartoshuk 2006).

Results also showed that there were proportionately more taste-responsive cells in the NTS of DIO vs. lean animals. This suggests the possibility that more NTS cells may be recruited to respond to taste to compensate for weakened taste responses. Obviously, the conversion of non-taste-responsive into taste-responsive cells would require that there were many NTS cells that had the requisite connectivity available but normally ineffective. Such potentiality of NTS cells in the short term has been shown following adaptation (Di Lorenzo and Lemon 2000) and following brief “pre-pulses” of tastants (Di Lorenzo et al. 2003) where taste responses appeared where none were previously. Changes in metabolic state, such as obesity, may unmask taste responses on a longer time scale.

Temporal Coding

As taste responses develop over time, the cumulative amount of information about stimulus identity conveyed by spike trains progressively increases in both lean and DIO animals. However, for traditional taste stimuli, this information was smaller for DIO compared with those in lean rats at all time points, and significantly smaller at 1 s and 2 s. This result may be another reflection of a weaker drive from the periphery (Kaufman et al., 2018). The shorter duration of responses in DIO rats implies that taste responses in these rats may trail off without conveying the amount of information that taste responses in their lean counterparts convey with longer lasting responses. Consistent with that idea is the observation that the information about taste

quality conveyed in DIO and lean rats was similar for the earlier parts of the responses but diverged at longer time intervals, where presumably information continued to accrue in lean rats but not in DIO subjects. Additionally, the proportion of neurons that use temporal coding to convey taste quality information was larger in lean rats than obese rats for both traditional and naturalistic stimuli. This result implies that NTS neurons in the DIO animal carry a disproportionate load of taste-related information compared with those in lean rats.

It can be argued that, unlike traditional taste stimuli, naturalistic tastants contain volatiles that provide olfactory input which stimulates and/or modulates NTS taste responses (Escanilla et al., 2015). The added olfactory information offered by naturalistic tastants may facilitate stimulus discrimination in both DIO and lean rats (Escanilla et al., 2015). This was apparent when animals were presented with a sweet stimulus at two different concentrations. There was no difference between NTS cells in DIO vs. lean rats in the amount of information discriminating two concentrations of sucrose. However, NTS cells in DIO animals were impaired in their ability to convey information about two different concentrations of grape juice, despite the fact that the sugar concentrations in the grape juice were matched to those of sucrose. It is possible that NTS cells in lean animals were able to take advantage of information from other modalities (e.g. olfaction) stimulated by grape juice but not sucrose, to discriminate different stimulus intensities, but NTS cells from DIO rats could not. This notion would be consistent with reports showing deficits in olfactory-driven behaviors in DIO rats (Lacroix et al. 2015).

Firing patterns in taste cells reflect behavior

In the gustatory cortex, the state of the neural network when information about a taste stimulus arrives is adjusted to optimize encoding of that stimulus (Yoshida and Katz 2011). Specifically, behavioral tasks that require active sensory acquisition modulate both pre-stimulus

activity and taste selectivity of single neurons (Yoshida and Katz 2011). Consistent with these results are data showing that cues that predict taste stimulation can lead to firing rate changes prior to taste presentation as well as a decrease in the taste response latency (Samuelson et al 2012; Fontanini et al. 2014). These data show that the animal's cognitive state, and therefore the state of the neural network encoding taste stimuli can influence the ability of neurons to convey taste-related information. In the present dataset, network activity both pre-sensory acquisition and during the acquisition phase was indexed by spontaneous and baseline firing rates respectively. Spontaneous firing rates did not differ significantly in NTS cells in DIO vs. lean rats; however, in lean rats, there was an increase in firing rate when the rats transitioned from spontaneous firing to sensory acquisition, where the rat was actively licking. A similar shift in firing rate did not occur in NTS cells in DIO rats, suggesting that the neural network in the NTS of DIO rats did not adjust to sensory acquisition as did the NTS in their lean counterparts. Moreover, when a lean animal initiated licking, firing rates in anti-lick cells were suppressed, offering taste-responsive cells a high signal-to-noise environment in which to convey information about taste stimuli. However, in DIO rats, there were proportionately fewer anti-lick cells than in lean rats, so taste responses in DIO rats, already relatively weak, occurred in a relatively noisy background compared with those in lean rats.

Lick-related activity is present throughout the rodent gustatory neuraxis (Stapleton et al. 2006; Gutierrez et al. 2010; Weiss et al. 2014), including the NTS (Roussin et al. 2012; Denman et al. 2019 *in press*), no doubt reflecting and influencing network-wide activity. The synchronous neural activity afforded by lick entrainment over several taste-related neural structures may enhance cross-structure communication (Fries 2015; Bonnefond et al. 2017). Lick-related activity in the NTS of the awake rat has been found to be widespread and informative with respect to taste quality discrimination (Denman et al. *in press*). Data reported here agree with

these results. However, NTS cells in DIO rats showed less lick coherence than those in lean rats. This result is consistent with the idea that lick-related activity enhances the neural representation of taste and that without it, taste stimuli are less well discriminated, as seen in the NTS of DIO rats reported here.

Conclusions

Present show that the neural processing of taste stimuli in the NTS of DIO rats is impaired. Taste responses in DIO rats are blunted and convey less information than those in lean rats. Shifts in the relative proportions of various patterns of ingestion-related firing may represent the system's adaptive mechanism aimed at compensating for a compromised taste system. The changes noted here may provide some insight into the mechanism(s) underlying altered taste perception in humans with obesity (Bartoshuk et al. 2006, Noel et al. 2017; Pepino et al. 2010, Sartor et al. 2011). Although there is evidence that weight loss improves taste perceptions in humans with obesity (Miras and le Roux 2010; Scruggs et al. 1994), it is unclear whether the changes found in the NTS of DIO animals can also be reversed. Further research will address this question.

References

- Baird JP, Travers JB, Travers SP (2001) Parametric analysis of gastric distension responses in the parabrachial nucleus. *AJP: Regulatory, Integrative and Comparative Physiology* 281:R1568–R1580
- Bartoshuk LM, Duffy VB, Hayes JE, Moskowitz HR, Snyder DJ (2006) Psychophysics of sweet and fat perception in obesity: problems, solutions and new perspectives. *Philosophical Transactions of the Royal Society B: Biological Sciences* 361:1137–1148.
- Beckman ME, Whitehead MC (1991) Intramedullary connections of the rostral nucleus of the solitary tract in the hamster. *Brain Research* 557:265–279.
- Berthoud H-R (2002) Multiple neural systems controlling food intake and body weight. *Neurosci Biobehav Rev* 26:393–428.
- Blouet C, Schwartz GJ (2012) Brainstem Nutrient Sensing in the Nucleus of the Solitary Tract Inhibits Feeding. *Cell Metabolism* 16:579–587.
- Bonnefond M, Kastner S, Jensen O (2017) Communication between Brain Areas Based on Nested Oscillations. *eNeuro* 4:ENEURO.0153–16.2017–34.
- Chandrashekar J, Hoon MA, Ryba NJP, Zuker CS (2006) The receptors and cells for mammalian taste. *Nature* 444:288–294.
- Cui RJ, Li X, Appleyard SM (2011) Ghrelin Inhibits Visceral Afferent Activation of Catecholamine Neurons in the Solitary Tract Nucleus. *Journal of Neuroscience* 31:3484–3492.

- Dando R (2015) The Plasticity of Taste Function Links the Appetitive Taste of Fats with Obesity. *Chem Percept* 8:53–60.
- Darcel N, Fromentin G, Raybould HE, Gougis S, Gietzen DW, Tomé D (2005) Fos-positive neurons are increased in the nucleus of the solitary tract and decreased in the ventromedial hypothalamus and amygdala by a high-protein diet in rats. *J Nutr* 135:1486–1490.
- Denman AJ, Sammons JD, Victor JD, Di Lorenzo PM (2018) Heterogeneity of neuronal responses in the nucleus of the solitary tract suggests sensorimotor integration in the neural code for taste. *J Neurophysiol* doi: 10.1152/jn.00578.2018.
- Di Lorenzo PM, Lemon CH. (2000) The neural code for taste in the nucleus of the solitary tract of the rat: effects of adaptation. *Brain Res.* 852(2):383-97.
- Di Lorenzo PM, Lemon CH, Reich CG. (2003) Dynamic coding of taste stimuli in the brainstem: effects of brief pulses of taste stimuli on subsequent taste responses. *J Neurosci.* 23(26): 8893-902.
- Duca FA, Swartz TD, Covasa M (2014) Effect of diet on preference and intake of sucrose in obese prone and resistant rats. McCutcheon JE, ed. *PLoS ONE* 9:e111232.
- Escanilla OD, Victor JD, Di Lorenzo PM (2015) Odor-taste convergence in the nucleus of the solitary tract of the awake freely licking rat. *J Neurosci* 35:6284–6297.
- Faulconbridge LF, Cummings DE, Kaplan JM, Grill HJ (2003) Hyperphagic effects of brainstem ghrelin administration. *Diabetes* 52:2260–2265.

- Fernandez-Garcia JC, Alcaide J, Santiago-Fernandez C, Roca-Rodriguez MM, Aguera Z, Baños R, Botella C, la Torre de R, Fernandez-Real JM, Fruhbeck G, Gomez-Ambrosi J, Jimenez-Murcia S, Menchon JM, Casanueva FF, Fernandez-Aranda F, Tinahones FJ, Garrido-Sanchez L (2017) An increase in visceral fat is associated with a decrease in the taste and olfactory capacity. *PLoS ONE* 12:e0171204–e0171214.
- Fries P (2015) Rhythms for Cognition: Communication through Coherence. *Neuron* 88:220–235.
- Ganchrow D, Ganchrow JR, Cicchini V, Bartel DL, Kaufman D, Girard D, Whitehead MC (2014) Nucleus of the solitary tract in the C57BL/6J mouse: Subnuclear parcellation, chorda tympani nerve projections, and brainstem connections. *Journal of Comparative Neurology* 522:1565–1596.
- Gardner MPH, Fontanini A (2014) Encoding and tracking of outcome-specific expectancy in the gustatory cortex of alert rats. *J Neurosci* 34:13000–13017.
- Giza BK, Scott TR (1983) Blood glucose selectively affects taste-evoked activity in rat nucleus tractus solitarius. *Physiology & Behavior* 31:643–650.
- Giza BK, Scott TR (1987) Intravenous insulin infusions in rats decrease gustatory-evoked responses to sugars. *Am J Physiol* 252:R994–R1002.
- Gutierrez R, Lobo MK, Zhang F, De Lecea L (2011) Neural integration of reward, arousal, and feeding: Recruitment of VTA, lateral hypothalamus, and ventral striatal neurons López-Casillas F, Vázquez-Prado J, eds. *IUBMB Life* 63:824–830.
- Gutierrez R, Simon SA, Nicolelis MAL (2010) Licking-induced synchrony in the taste-reward circuit improves cue discrimination during learning. *J Neurosci* 30:287–303.

- Hajnal A, Covasa M, Bello NT (2005) Altered taste sensitivity in obese, prediabetic OLETF rats lacking CCK-1 receptors. *AJP: Regulatory, Integrative and Comparative Physiology* 289:R1675–R1686.
- Karimnamazi H, Travers SP, Travers JB (2002) Oral and gastric input to the parabrachial nucleus of the rat. *Brain Research* 957:193–206.
- Kaufman A, Choo E, Koh A, Dando R (2018) Inflammation arising from obesity reduces taste bud abundance and inhibits renewal Cadwell K, ed. *PLoS Biol* 16:e2001959–14.
- Kovacs P, Hajnal A (2008) Altered pontine taste processing in a rat model of obesity. *Journal of Neurophysiology* 100:2145–2157.
- Lacroix M-C, Caillol M, Durieux D, Monnerie R, Grebert D, Pellerin L, Repond C, Tolle V, Zizzari P, Baly C (2015) Long-Lasting Metabolic Imbalance Related to Obesity Alters Olfactory Tissue Homeostasis and Impairs Olfactory-Driven Behaviors. *Chem Senses* 40:537–556.
- Miras AD, le Roux CW (2010) Bariatric surgery and taste: novel mechanisms of weight loss. *Current Opinion in Gastroenterology* 26:140–145
- Noel CA, Sugrue M, Dando R (2017) Participants with pharmacologically impaired taste function seek out more intense, higher calorie stimuli. *Appetite* 117:74–81.
- Ogden CL, Carroll MD, Fryar CD, Flegal KM (2015) Prevalence of Obesity Among Adults and Youth: United States, 2011-2014. *NCHS Data Brief*:1–8.

Pepino MY, Finkbeiner S, Beauchamp GK, Mennella JA (2010) Obese Women Have Lower Monosodium Glutamate Taste Sensitivity and Prefer Higher Concentrations Than Do Normal-weight Women. *Obesity* 18:959–965.

Purpura KP, Victor JD (1997) Sensory Coding in Cortical Neurons. *Annals of the New York Academy of Sciences* 835:330–352

Ritter S, Dinh TT, Zhang Y (2000) Localization of hindbrain glucoreceptive sites controlling food intake and blood glucose. *Brain Research* 856:37–47.

Roussin AT, D'Agostino AE, Fooden AM, Victor JD, Di Lorenzo PM (2012a) Taste coding in the nucleus of the solitary tract of the awake, freely licking rat. *J Neurosci* 32:10494–10506.

Roussin AT, D'Agostino AE, Fooden AM, Victor JD, Di Lorenzo PM (2012b) Taste coding in the nucleus of the solitary tract of the awake, freely licking rat. *J Neurosci* 32:10494–10506.

Rozengurt E (2006) Taste Receptors in the Gastrointestinal Tract. I. Bitter taste receptors and α -gustducin in the mammalian gut. *American Journal of Physiology - Gastrointestinal and Liver Physiology* 291:G171–G177

Sammons JD, Weiss MS, Victor JD, Di Lorenzo PM (2016) Taste coding of complex naturalistic taste stimuli and traditional taste stimuli in the parabrachial pons of the awake, freely licking rat. *Journal of Neurophysiology* 116:171–182.

Samuelsen CL, Gardner MPH, Fontanini A (2012) Effects of Cue-Triggered Expectation on Cortical Processing of Taste. *Neuron* 74:410–422.

- Sartor F, Donaldson LF, Markland DA, Loveday H, Jackson MJ, Kubis H-P (2011) Taste perception and implicit attitude toward sweet related to body mass index and soft drink supplementation. *Appetite* 57:237–246.
- Scott TR, Mark GP (1987) The taste system encodes stimulus toxicity. *Brain Research* 414:197–203.
- Scruggs D, Buffington C, Cowan G (1994) Taste Acuity of the Morbidly Obese before and after Gastric Bypass Surgery. *Obes Surg* 4:24–28.
- Shin AC, Townsend RL, Patterson LM, Berthoud H-R (2011) "Liking" and "wanting" of sweet and oily food stimuli as affected by high-fat diet-induced obesity, weight loss, leptin, and genetic predisposition. *Am J Physiol Regul Integr Comp Physiol* 301:R1267–R1280
- Spector AC, Spector AC, Travers SP (2005) The Representation of Taste Quality in the Mammalian Nervous System. *Behavioral and Cognitive Neuroscience Reviews* 4:143–191
- Stapleton JR, Lavine ML, Wolpert RL, Nicolelis MAL, Simon SA (2006) Rapid taste responses in the gustatory cortex during licking. *J Neurosci* 26:4126–4138.
- Travers S, Breza J, Harley J, Zhu J, Travers J (2018) Neurons with diverse phenotypes project from the caudal to the rostral nucleus of the solitary tract. *Journal of Comparative Neurology* 526:2319–2338.
- Victor JD, Purpura KP (1996) Nature and precision of temporal coding in visual cortex: a metric-space analysis. *Journal of Neurophysiology* 76:1310–1326.

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

Whitehead MC, Bergula A, Holliday K (2000) Forebrain projections to the rostral nucleus of the solitary tract in the hamster. *J Comp Neurol* 422:429–447.

Yoshida T, Katz DB (2011) Control of prestimulus activity related to improved sensory coding within a discrimination task. *J Neurosci* 31:4101–4112.

Zhan C, Zhou J, Feng Q, Zhang J-E, Lin S, Bao J, Wu P, Luo M (2013) Acute and long-term suppression of feeding behavior by POMC neurons in the brainstem and hypothalamus, respectively. *J Neurosci* 33:3624–3632.

Zhang X-J, Wang Y-Q, Long Y, Wang L, Li Y, Gao F-B, Tian H-M (2013) Alteration of sweet taste in high-fat diet induced obese rats after 4 weeks treatment with exenatide. *Peptides* 47:115–123.

Zocchi D, Wennemuth G, Oka Y (2017) The cellular mechanism for water detection in the mammalian taste system. *Nat Neurosci* 20:927–933.

Figure 1. Body Composition. A. Scattergram of body composition data from lean and DIO rats. Horizontal lines indicate the mean and error bars represent SEM. Each dot indicates an animal's fat mass in grams, lean and bone mass content in grams, total weight (in grams; left Y-Axis), and body fat percentage (right Y-Axis). B. DXA scan images of a lean and obese rat.

Figure 2. Examples of taste responses on different time scales in NTS. A. An example of a taste-responsive NTS neuron with a 5-lick response to taste. The first lick of the 5-lick stimulus trail is at zero; time bins are 100 ms. B. An example of a taste-responsive NTS neuron with a lick-by-lick response. Each individual stimulus lick is positioned at zero; time bins are 5 ms. For both A. and B., each panel shows rasters (top) and peristimulus time histograms (PSTHs; bottom). Colored triangles in the rasters indicate a lick reinforced with stimulus.

Figure 3. Spontaneous and baseline firing rates in NTS cells. A. Mean firing rates are in spikes/sec $\pm$ SEM. Spontaneous firing rates are from 10 s devoid of licks. B) Scatterplots of spontaneous firing rate plotted against baseline firing rate for each cell in lean (top) and DIO (bottom) rats.

Figure 4. Examples of firing patterns of NTS cells. A. Raster and PSTH of a neuron's firing profile during licking. Each lick is positioned at $t = 0$; time bin = 10 ms. This cell increases its firing rate just before the occurrence of each lick. B. Rasters and PSTHs of an anti-lick cell. The zero point is positioned just before the beginning of a lick bout (defined as at least 1 s of continuous licking), upper, or at the end of a lick bout, lower. Time bin = 100 ms. This cell shows a lower firing rate during a lick bout and a higher firing rate when the rat is not licking. C. Rasters and PSTHs of a lick bout cell. The zero point is positioned just before the beginning of a lick bout, upper, or at the end of a lick bout, lower. This cell shows a higher firing rate during a lick bout and a lower firing rate when the rat is not licking. In A.-C., each green triangle indicates the occurrence of a lick.

Figure 5. Response and tuning characteristics of NTS cells. A. Proportion of NTS cells with various patterns of firing with respect to taste stimulus presentation and licking behavior. There is a larger proportion of taste-responsive cells in DIO rats compared to lean rats and a smaller proportion of anti-lick cells. B. Tuning characteristics of taste-responsive NTS cells in lean and DIO rats. Shown is the proportion of cells that respond to one, two, three or four traditional tastants (0.1 M sucrose, 0.05 M NaCl, 0.016 M citric acid, 0.002 M caffeine). Cells with 5-lick or lick-by-lick responses are collapsed.

Figure 6. Lick coherence in taste-responsive, lick, and lick-anti-lick mix cells. Mean and individual values from each cell with significant lick coherence within the 4-9 Hz range are shown. See text for details.

Figure 7. Proportions of 5-lick and lick-by-lick responses across taste stimuli for DIO and lean rats. A. Traditional taste stimuli. B. Naturalistic taste stimuli. Abbreviations are as follows: Lo Su, 0.1 M sucrose; Hi Su, 0.5 M sucrose; NaCl, 0.05M NaCl; Citric Acid, 0.01 M citric acid; Caff, 0.002 M caffeine; AS, artificial saliva; Hi GJ, 25% grape juice; Lo GJ, 100% grape juice; Clm J, clam juice; Lm J, lemon juice; Coff, coffee; Cream, 25% heavy cream.

Figure 8. 5-lick response magnitudes (in spikes per sec; sps) in DIO and lean rats. Baseline firing rates have been subtracted from stimulus-evoked firing rates. A. Traditional taste stimuli. B. Naturalistic taste stimuli. For each stimulus, response magnitudes of individual cells are superimposed on the mean values. Abbreviations are as in Figure 7.

Figure 9. Lick-by-lick response magnitudes (in sps) for DIO and lean rats. Baseline firing rates have been subtracted from stimulus-evoked firing rates. For each stimulus, response magnitudes of individual cells are superimposed on the mean values. Abbreviations are as in Figure 7.

5-lick response duration

Lick-by-lick response duration

Figure 10. Taste response durations in NTS cells in DIO and lean rats. For each stimulus, response magnitudes of individual cells are superimposed on the mean values. Abbreviations are as in Figure 7.

Figure 11. Latencies of 5-lick taste responses in NTS cells in DIO and lean rats. The percentile of each response is plotted against its latency. Abbreviations are as in Figure 7.

Figure 12. Latencies of lick-by-lick taste responses in NTS cells in DIO and lean rats. The percentile of each response is plotted against its latency. Abbreviations are as in Figure 7.

Figure 13. Examples of lick-by-lick taste responses in one unit from a lean rat (left) and another from a DIO rat (right). Top of each panel shows a raster; bottom of each panel shows a PSTH. Colored triangles indicate reinforced licks. Inset shows waveforms.

Figure 14. Results of metric space analysis of taste responses in DIO and lean rats. Results for taste-responsive cells are shown in top graphs for traditional (left) and naturalistic (right) stimuli. Similar results from lick cells are shown on bottom. Traditional taste stimuli included in the analyses were 0.1 M sucrose, 0.05 M NaCl, 0.01 M citric acid and 0.002 M caffeine; naturalistic taste stimuli included were 25% grape juice, clam juice, lemon juice and coffee. Percentages near each data point indicate the proportion of responses that conveyed a significant amount of information about taste quality ($H_{max} > H_{shuffled} + 2SDs$). Perfect discrimination = 2 bits.

Figure 15. Results of metric space analysis of taste responses to high and low concentrations of sweet stimuli. Traditional taste stimuli included in the analyses were 0.1 M and 0.5 M sucrose; naturalistic stimuli included in the analyses included 25% and 100% grape juice. Perfect discrimination = 1 bit. Percentages near each data point indicate the proportion of responses that conveyed a significant amount of information about sweet stimulus intensity ($H_{max} > H_{shuffled} + 2SDs$).

Figure 16. Results of histological analyses reconstructing recording locations in lean and DIO rats. Photomicrographs of coronal sections through the rat brainstem. Dashed lines indicate the area of the NTS. Stars indicate lesion site, red = DIO rats, blue = lean rats. Abbreviations as follows: Sp5, Spinal trigeminal nucleus; PCRt, parvocellular reticular nucleus; 4V, 4th ventricle; ION, inferior olivary nucleus; MeV, medial vestibular nucleus; Mlf, medial longitudinal fasciculus; icp, inferior cerebellar peduncle.