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Two types of inhibitory influences target different groups of taste-responsive cells in the nucleus of the solitary tract of the rat

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Abstract

Electrical stimulation of the chorda tympani nerve (CT; innervating taste buds on the rostral tongue) is known to initiate recurrent inhibition in cells in the nucleus of the solitary tract (NTS, the first central relay in the gustatory system). Here, we explored the relationship between inhibitory circuits and the breadth of tuning of taste-responsive NTS neurons. Initially, NTS cells with evoked responses to electrical stimulation of the CT (0.1 ms pulses; 1 Hz) were tested with each of four tastants (0.1 M NaCl, 0.01 M HCl, 0.01 M quinine and 0.5 M sucrose) in separate trials. Next, the CT was electrically stimulated using a paired-pulse (10–2000 ms interpulse interval; blocks of 100 trials) paradigm. Forty-five (30 taste-responsive) of 51 cells with CT-evoked responses (36 taste-responsive) were tested with paired pulses. The majority (34; 75.6%) showed paired-pulse attenuation, defined as fewer evoked spikes in response to the second (test) pulse compared with the first (conditioning) pulse. A bimodal distribution of the peak of paired-pulse attenuation was found with modes at 10 ms and 50 ms in separate groups of cells. Cells with early peak attenuation showed short CT-evoked response latencies and large responses to relatively few taste stimuli. Conversely, cells with late peak attenuation showed long CT-evoked response latencies and small taste responses with less selectivity. Results suggest that the breadth of tuning of an NTS cell may result from the combination of the sensitivities of peripheral nerve inputs and the recurrent influences generated by the circuitry of the NTS.

Keywords

taste; gustation; chorda tympani nerve; nucleus of the solitary tract; electrical stimulation; inhibition

1. Introduction

The primary function of the taste system is to identify and promote ingestion of nutrients and to avoid ingestion of toxins. To accomplish this, an organism must be able to discriminate among various taste stimuli. It has been argued that this function is in part subserved by the chorda tympani nerve (CT; a branch of the facial nerve that innervates taste buds on the anterior two-thirds of the tongue; Geran et al., 2002;) since cutting this nerve severely disrupts discriminative ability in rats (Spector and Grill, 1992). The CT nerve sends projections to the nucleus of the solitary tract (NTS) where taste information converges with mechanical and thermal (e.g. Ogawa et al., 1984; Travers and Norgren, 1995) input. Since the NTS is the first

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relay in the central gustatory pathway, an understanding of the response properties of these cells in relation to CT nerve input is important for an appreciation of how taste might be encoded in the brain.

It is well known that taste-responsive cells in the NTS are more broadly tuned than CT fibers that project to them (see Norgren, 1983; Spector and Travers, 2005). This is likely the result of the divergence of CT fibers as they make contact with NTS cells (see Lundy and Norgren, 2004). However, considering the many observations that the breadth of tuning of NTS cells can change with conditions such as sodium deprivation (McCaughey et al., 1996), conditioned taste aversion learning (McCaughey et al., 1997), changes in sex hormones (Di Lorenzo and Monroe, 1989, 1990), it is likely that the breadth of tuning of a given NTS cell is a dynamic feature of the cell and is the end result of a balance of both excitatory and inhibitory influences (Di Lorenzo, 2000; Grabauskas and Bradley, 1998, 2003).

The CT makes excitatory connections on NTS cells (Smith et al., 1998); however, electrical stimulation of this nerve evokes inhibition as well (Lemon and Di Lorenzo, 2002; Bradley and Grabauskas, 1998). Studies have suggested that inhibition initiated by CT nerve stimulation may mediate both temporal and spatial encoding of taste stimuli (Lemon and Di Lorenzo, 2002), the tuning of individual taste neurons (Lemon and Di Lorenzo, 2002) and synaptic plasticity within the NTS (Bradley and Grabauskas, 1998; Grabauskas and Bradley, 1998; 1999; 2003). Evidence suggests that this inhibition is mediated by GABAergic interneurons within the NTS that likely play a critical role in the processing of taste information (Smith and Li, 1998; Grabauskas and Bradley, 1999). In fact, Smith and Li (1998) have provided evidence that GABAergic inhibition may function to narrow the breadth of tuning of taste-responsive cells in the NTS.

Electrophysiological studies of the effects of CT stimulation on NTS cells have also suggested that inhibition arising from CT stimulation may shape the tuning profile of taste-responsive NTS cells. Based on a report by Grabauskas and Bradley (1998) showing that tetanic stimulation of the solitary tract *in vitro* potentiates inhibitory postsynaptic potentials in the rostral NTS, we hypothesized that tetanic stimulation of the CT nerve *in vivo* might affect responses to taste stimuli presented shortly thereafter (Lemon and Di Lorenzo, 2002). Tetanic stimulation of the CT nerve at frequencies approximating naturally occurring volleys produced a predominantly suppressive effect on taste responses, most often to quinine, in NTS cells. To assess whether CT stimulation-induced inhibition has any functional relevance to neural processing in a natural setting, we next presented brief (100 ms) pulses of taste stimuli just prior to a lengthier presentation of the same or a different tastant (Di Lorenzo et al., 2003). The “prepulses” of taste stimuli were meant to emulate the tetanic stimulation of the CT nerve. Results showed that the effects of these prepulses on subsequent taste responses differed according to the breadth of tuning of the cell. That is, the extent to which a cell responded broadly to stimuli representing the basic taste qualities (sweet, sour, salty or bitter) was predictive of whether prepulses changed the magnitude and temporal organization of responses to subsequently presented taste stimuli. Broadly tuned NTS cells were more likely to be affected by taste prepulses than cells that were more narrowly tuned. Collectively, these results suggest that the way that NTS cells are affected by inhibition may predict whether they are broadly or narrowly tuned across taste stimuli.

To investigate this idea, we examined the time course of CT-generated inhibition and its relationship to the breadth of tuning of NTS cells. The CT was electrically stimulated with paired pulses at various interpulse intervals (IPIs) under the assumption that if the first conditioning pulse generated a recurrent inhibitory influence, test pulses that occurred shortly thereafter would evoke fewer spikes. Varying the IPI provided a way to study the strength and duration of the inhibition. Previous studies have confirmed the existence of such time-

dependent inhibition in the NTS both *in vivo* (Toney and Mifflin, 1994; Scheuer, et al., 1996) and *in vitro* (Grabauskas and Bradley, 2003); however the correlation of this inhibition with other functional properties has not been studied. Results of the present study provide evidence for two types of inhibitory influences on NTS cells following CT stimulation. One type follows CT stimulation with a short latency, fades rapidly and is found in cells with large responses to relatively few taste stimuli. A second type peaks in strength later than the first type and fades more gradually. It was found in NTS cells that showed broad tuning but weak taste responses.

2. Results

2.1. General response characteristics

Fifty-one NTS cells with evoked responses to CT stimulation were recorded. Of these, 36 cells responded to taste stimuli. The remaining 15 cells showed evoked responses to CT stimulation but no response to any of the tastants tested. Effects of paired-pulse electrical stimulation of the CT nerve were recorded in 45 cells, of which 30 (66.7%) were responsive to taste stimulation. Cells that responded to CT stimulation generally showed low spontaneous rates of firing; mean spontaneous rate = 1.74 ± 0.36 SEM spikes per sec (sps); taste-responsive cells showed higher spontaneous firing rates (mean = 2.11 ± 0.48 sps) than non-taste responsive cells (mean = 0.83 ± 0.28 sps). This difference was not, however, statistically significant.

Those cells that were responsive to taste stimuli generally responded to more than one of the tastants tested. Six cells responded to all four taste stimuli, 17 responded to three stimuli, 5 responded to two stimuli and 8 responded to only one taste stimulus. When cells were classified according to their “best” stimulus, defined by the tastant that evoked the highest magnitude of response, 20 (of 36, 55.6%) were NaCl best, 7 (19.4%) were sucrose best, 7 (19.4%) were HCl best and 2 (5.6%) were quinine best. The average Uncertainty measure, an index of breadth of tuning across tastants (Smith and Travers, 1979), was 0.69 ± 0.04 . In addition to the Uncertainty measure, we calculated a second metric, called “Selectivity” (*S*), to characterize taste-responsive cells by both their magnitude of response and breadth of tuning. Among the 36 taste-responsive cells in our sample, the mean Selectivity value was 10.3 ± 1.4 sps (range = 0.8 to 31.1 sps; median = 7 sps). Interestingly, cells that showed high Selectivity to taste stimuli had shorter latencies of CT-evoked responses ($r = -0.36$, $p = 0.03$) and higher spontaneous rates of firing ($r = 0.67$, $p < 0.0001$).

2.2. Effects of CT nerve stimulation on NTS cells

The majority of cells responded to electric shocks delivered to the CT nerve with a single time-locked spike; however, four cells responded to CT stimulation with multiple evoked spikes. Three of these cells showed two spikes and one cell showed three spikes following electrical CT stimulation. The latency of response between the primary and secondary spikes ranged from 3 to 5 ms. Figure 1 shows the distribution of CT-evoked response latencies for all 51 cells. The mean latency of response to CT stimulation was 10.9 ± 1.2 ms (median = 7.5 ms). Taste-responsive cells showed significantly shorter CT-evoked response latencies ($n = 36$; mean = 9.3 ± 1.2 ms; median = 5 ms) than non-taste-responsive cells ($n = 15$; mean = 15.1 ± 2.8 ms; median = 11 ms; $t(49) = 2.2$, $p < 0.05$). Cells that showed evoked responses to CT nerve stimulation showed a mean latency variability of 4.0 ± 0.3 ms. Cells with the longest latencies showed the largest latency variability, as evidenced by a significant correlation between the latency of evoked response and latency variability ($r = 0.88$, $p < 0.01$).

2.3. Effects of paired-pulse electrical stimulation of the CT nerve

Paired-pulse stimulation of the CT nerve was analyzed in 45 cells (30 taste-responsive and 15 non-taste-responsive). Of these, 34 cells (75.6%) showed paired-pulse attenuation. These included both taste-responsive ($n = 23$) and non-taste-responsive ($n = 11$) cells. The remaining

nine of the 45 cells (20%) showed neither attenuation nor enhancement to paired-pulse stimulation. Six of these cells were taste-responsive. Two cells showed enhancement (1 taste-responsive); however in both cases enhancement was either preceded or followed by attenuation. Figure 2 shows the paired-pulse enhancement and attenuation exhibited by one cell at different inter-pulse intervals. After an IPI of 500 msec there was no evidence of paired-pulse attenuation or enhancement in any cell.

2.4. Bimodal distribution of peak paired-pulse attenuation

Paired-pulse attenuation in NTS cells was found to either peak early with a rapid decay or to peak late with a prolonged decay. Figure 3 shows the distribution of the peak of paired-pulse attenuation across taste-responsive and non-taste-responsive cells. This distribution was bimodal for taste-responsive cells with modes at 10 ms (12 cells; 35.3%) and 50 ms (13 cells; 38.2%). Although the distribution of peak attenuation for taste-responsive cells was bimodal, the corresponding distribution for non-taste-responsive cells was skewed toward late peak attenuation. Figure 4 shows the time course of attenuation for all cells with early peak attenuation (≤ 20 ms) or with late peak attenuation (≥ 30 ms). The interpolated average decay time constant of attenuation in cells with early peak attenuation was 61 ± 18.7 ms while the decay time constant in cells with delayed peak attenuation was 300 ± 51 ms. Mean decay time constants of attenuation for taste-responsive cells ($n = 23$; 150 ± 39.5 ms) were significantly shorter ($t(32) = -2.23$, $p < 0.05$) than those for non-taste-responsive cells ($n = 11$; 315 ± 68.6 ms).

When cells were divided into those with early peak attenuation vs. late peak attenuation, other functional correlates were apparent. For example, cells with earlier peaks were more selective to taste stimuli (Spearman $\rho = -0.64$, $p < 0.01$) and had shorter latencies of response to CT stimulation (Spearman $\rho = 0.73$, $p < 0.01$). Because NaCl best cells are typically more selective than HCl or quinine best cells, it is not surprising that almost all (9 of 10) NaCl best cells but almost none of the HCl and quinine best cells showed early peak attenuation. Sucrose best cells were evenly divided between early and later peak attenuation. Figure 5 shows the distribution of best stimuli for cells that showed early and late peak attenuation.

2.5. Location of NTS cells

The locations of 36 NTS cells (27 taste-responsive; 9 non-taste-responsive) were reconstructed following histological analyses (see Figure 6). The majority of cells showing evoked responses to CT stimulation were located in the rostral central and rostral lateral NTS. There were no differences in the locations of taste-responsive and non-taste-responsive neurons that showed evoked responses to CT stimulation.

3. Discussion

The main finding of the present investigation is the discovery of two types of inhibitory influences on NTS cells following electrical stimulation of the CT nerve. These inhibitory influences had different time courses and were associated with different patterns of taste responsiveness. Twelve NTS cells showed an early peak in paired-pulse attenuation at ~ 10 ms that decayed rapidly over the course of ~ 100 ms. These cells showed short latencies of CT-evoked responses and high Selectivity across taste stimuli. Conversely, thirteen cells showed paired-pulse attenuation that peaked at ~ 50 ms and decayed slowly over ~ 500 msec. These cells responded to CT stimulation at relatively long and more variable latencies and were less selective in their responses across taste stimuli. Results support the idea that the particular compliment of taste sensitivity of an NTS cell may result from the combination of the sensitivities of peripheral nerve inputs and the recurrent influences generated by the circuitry of the NTS. Furthermore, present results suggest that narrowly tuned taste-responsive NTS

cells with large response magnitudes transmit CT input with higher fidelity (short latency responses that produce short-lived inhibition) than more broadly tuned cells (long and variable latency responses that produce long lasting inhibition).

3.1. Evoked responses to CT stimulation

Electrophysiological responses to electrical stimulation of the CT nerve were obtained from 51 cells in the NTS. The mean latency of evoked response for taste-responsive cells ($n = 36$) in the present study was 9.3 ± 1.2 ms. This was comparable to the mean latency reported by Ogawa and Kaisaku (1982) (7.2 ± 4.3 ms) but somewhat longer than that reported by Lemon and Di Lorenzo (2002) (3.8 ± 0.1 ms). However, in the latter study latencies of response longer than 20 ms could not be detected, so the mean CT response latency was likely skewed toward a low value. The latency reported here is also longer than the latency reported by Grabauskas and Bradley (1996; 4.8 ± 0.6 ms) in an *in vitro* preparation; however, their point of CT stimulation was presumably closer to the target NTS cell than was the case in the present study.

3.2. Non-taste-responsive cells with CT-evoked responses

Curiously, almost a third (15 of 51; 29.4%) of the NTS cells with CT-evoked responses recorded in the present study did not respond to any of the tastants tested. These cells showed generally low spontaneous rates of activity (0.83 ± 0.28 sps) and long latencies of evoked responses to CT stimulation (15.1 ± 2.8 ms). Because of these long latencies and relatively large variability of response latency (mean = 4.9 ± 0.6 ms), it is likely that many of these non-taste-responsive cells receive polysynaptic input from the CT nerve (see Miles, 1986). Alternatively, because some properties of the non-taste-responsive NTS cells were similar to those of cells with evoked response to glossopharyngeal nerve stimulation observed by Hallock and Di Lorenzo (2006), i.e. low spontaneous rates (1.15 ± 0.31 sps) and long latencies of GP-evoked response (18.0 ± 1.32 ms), it is tempting to suggest that they actually receive convergent CT and GP input. Such convergence may function to mediate appropriate oromotor behavior associated with taste stimulation (St. John and Spector, 1998). In addition, many studies have shown that some NTS cells are responsive to thermal and mechanical stimuli (e.g. Ogawa et al., 1984; Travers and Norgren, 1995), suggesting the possibility that the non-taste-responsive cells were responsive to other sensory modalities.

3.3. Modes of paired-pulse attenuation

The current study revealed two distinct time-courses of paired pulse attenuation: one that peaked and degraded relatively rapidly, and a second that peaked later and degraded more slowly. There could be several factors that might account for these observations. For example, potential mechanisms for attenuation might include recurrent intranuclear inhibition or inhibitory input from other structures. Alternatively, paired pulse attenuation might be the result of frequency-dependent changes at the afferent nerve-NTS synapse (Doyle and Andresen, 2001; Miles, 1986). Whatever the mechanism, there are several reports that suggest that peripheral nerve input generates an inhibitory influence in NTS cells. For example, Grabauskas and Bradley (2003) have shown that electrical stimulation of the solitary tract produces inhibitory postsynaptic currents (IPSCs) in NTS cells *in vitro*. Field potential recordings in the NTS of anesthetized rats following paired-pulse stimulation of the CT nerve have also provided evidence for CT-initiated inhibition (Lemon and Di Lorenzo, 2002). Similarly, brief pulses of taste stimuli presented one sec (but not 5 sec) prior to a subsequent taste stimulus have been shown to suppress the response to the second tastant (Di Lorenzo et al., 2003), implying that the brief “prepulse” generated an inhibitory influence. Interestingly, those cells that showed this effect were more broadly tuned across taste stimuli and may correspond to NTS cells described in the present study that showed long latencies to CT stimulation and a slow time course of paired-pulse attenuation.

There is also some evidence from *in vitro* experiments that support the idea that there are two types of inhibitory influences on NTS cells that differ in their time courses of decay. For example, Grabauskas and Bradley (2003) described two types of IPSCs in NTS cells following electrical stimulation of the solitary tract. These two IPSCs differed in their decay time constants: the majority of cells showed IPSCs with decay time constants of 38 and 181 ms, while the remaining cells showed IPSCs with one decay constant at 59 ms. Consistent with these results, estimates of the decay time constants of paired pulse attenuation for the two types of NTS cells in the present study are 61 ms and 300 ms.

Neurochemical underpinnings for inhibition in the gustatory NTS include the involvement of several neurotransmitter systems. For example, it is well known that cells of the rostral NTS show tonic GABAergic inhibition, largely mediated by GABA_A receptors (Liu et al., 1993; Smith and Li, 1998; Wang and Bradley, 1993). In addition, Uteshev and Smith (2006) demonstrated the involvement of both voltage-gated potassium A-channels and hyperpolarization-activated potassium/sodium channels in mediating inhibitory responsiveness to acetylcholine (ACh) in the rostral NTS. Voltage-gated inhibition, mediated by potassium A-channels was shown to result in a transient hyperpolarization of rostral NTS neurons which may increase the firing threshold and slow the initiation of the action potential. This study also demonstrated the importance of the m2-type ACh receptor that produces ligand-gated inhibition through its association with inward-rectifier potassium channels. The activation of this channel has been shown to produce inhibition by shunting current across the membrane. Endogenous opioids may also mediate inhibitory control of taste processing in the rostral NTS (Li et al., 2003; Davis and Kream, 1993). Li et al. (2003) showed that microinjection of met-enkephalin into the gustatory NTS suppresses taste responsivity. Met-enkephalin also completely blocked spontaneous activity and spiking from anodal electrical stimulation of the anterior tongue in some NTS cells, indicating a strong role for the opioid system in modulating information from CT afferents (Li et al., 2003).

In addition to inhibition that is intrinsic to the NTS, inhibition may also arise from neural structures that project to the NTS. Gustatory neurons in the rostral NTS receive both local inhibitory connections and inhibitory input from higher structures such as the parabrachial nucleus of the pons (Di Lorenzo and Monroe, 1995, 1997), the venteroposterior thalamus (Cho et al., 2008) and the central nucleus of the amygdala (Cho et al., 2003).

In the present study, nine NTS cells did not show any evidence of paired pulse attenuation. In a similar study of paired pulse stimulation of the aortic nerve in the caudal NTS, Scheuer et al. (1996) also found evidence of paired pulse inhibition, but only in cells that received aortic nerve input that was polysynaptic. In that study, they examined the percent attenuation at an IPI of 50 msec and showed that the preponderance of monosynaptically activated cells were unaffected by paired pulse stimulation but the opposite was true for polysynaptically activated cells. Consistent with these results, at an IPI of 50 msec, in the present study the average latency of NTS cells that showed less than 10% paired pulse attenuation was 6.6 ± 1.9 msec (mean variability of latency = 2.8 ± 0.6 msec); conversely, the average latency of NTS cells that showed over 90% paired pulse attenuation at an IPI of 50 msec was 19.5 ± 2.2 msec (mean variability of latency = 5.8 ± 0.4 msec). In addition, among the nine cells that showed no evidence of paired pulse attenuation, the average latency of response to CT stimulation was 7.4 ± 1.9 sec (mean variability of latency = 3.1 ± 0.6 msec). Although we cannot with certainty predict which of the cells that we recorded were mono- or polysynaptically activated by CT stimulation, these results point to the idea that monosynaptic activation by the CT nerve may be less likely to produce a recurrent inhibition in NTS cells. On the other hand, there were many cells with short latencies of CT response that showed ample evidence of paired pulse attenuation, mostly with an early peak.

3.4. Implications of the Time Course of Inhibition for Taste Coding in the NTS

The observation of two types of inhibitory influences on taste-responsive NTS cells has at least two implications for the study of taste coding. First, the correlation of the time course of inhibition with breadth of tuning suggests that this characteristic of taste-responsive cells in the NTS may be the result of neural circuitry that is different in narrowly and broadly tuned NTS cells. Other functional differences between broadly and narrowly tuned cells have also been noted previous studies (Di Lorenzo et al., 2003; Di Lorenzo and Victor, 2003). A second implication of the present results may relate to temporal coding. That is, in narrowly tuned cells with brief recurrent inhibition, incoming signals may be transmitted upstream with relatively high fidelity. Because of their relatively short latencies of responses to CT stimulation, it is possible that these cells receive monosynaptic input from the CT nerve. In contrast, the slow time course of inhibition in broadly tuned cells may serve to stabilize the temporal pattern of the taste-evoked spike train by acting as a low pass filter. Consistent with this notion is the observation that spike timing conveys information about taste quality most frequently in broadly tuned cells in the NTS (Di Lorenzo and Victor, 2003). In addition, a longer time course of inhibition may modulate the background activity, i.e. the “noise” level, against which relay neurons carry their message forward.

4. Experimental Procedures

Experiments were carried out on 37 adult male Sprague-Dawley rats (350–450 g). All animals were housed in pairs in plastic cages and kept on a 12-hour light/dark cycle with lights on at 7:00 AM. Food and water were provided *ad libitum*. Animals were cared for according to the requirements of the Institutional Animal Care and Use Committee of Binghamton University.

4.1. Surgery

Prior to surgery rats were deeply anesthetized with urethane (1.5g/kg, i.p.). Body temperature was held constant at 37° C by a heating pad. Rats were tracheotomized to facilitate breathing during taste stimulus delivery and their head was mounted in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA). The animal's head was fixed with the incisor bar at 5 mm below the interaural line. The skull was exposed and stainless steel screws were implanted just anterior to bregma. A non-traumatic head holder was secured with dental cement to the stainless steel screws implanted in the skull. The pinna of the left ear was removed to expose the ear canal and tympanic membrane. Stimulating electrodes (platinum, 0.003” diam.) were inserted in the tympanic membrane on either side of the malleus to allow for the passage of electrical current across the chorda tympani nerve. The occipital bone and meninges were removed and the posterior cerebellum was gently aspirated to allow access to the caudal medulla.

4.2. Electrophysiological recording

The NTS was located approximately 2.7 mm rostral and 1.8 mm lateral to the obex and 1.0 mm from the dorsal surface of the brain. An etched tungsten microelectrode (FHC Inc., Bowdoinham, ME), insulated except for the tip (18–20 M Ω , 1 V at 1 kHz) was used for electrophysiological recording. All recorded activity was digitized with an analogue-to-digital interface (Model 1401, Cambridge Electronic Designs, Cambridge, UK) and stored on a computer. Spike2 software (Cambridge Electronic Designs) was used to analyze the data. Principal component analyses and template matching based on waveform parameters were used offline to assess cell isolation. A signal to noise ratio of 3:1 was required. The precise timing of cell spiking with 1 ms precision could be analyzed with respect to the timing of both tastant delivery and each pulse of electrical stimulation.

4.3. Experimental Protocol

As the electrode was lowered, the taste-responsive portion of the NTS was identified by the presence of a response in the background to presentation of NaCl (0.1 M) trial, as described below, and/or an evoked response to electrical stimulation of the CT nerve (0.1 ms, 0.5 mA, 1 Hz). When an NTS cell was isolated, 2 min of spontaneous activity was recorded. Taste stimuli were then presented in individual trials. Next, the CT nerve was stimulated at 1 Hz while gradually increasing the current to a level that reliably evoked a spike. This current level was used for the remainder of the experiment. To determine the latency of response, 100 pulses were presented at 1 Hz. Paired-pulse stimulation of the CT nerve proceeded in blocks of 100 presentations. Pulses were delivered at IPIs of 10, 20, 30, 50, 100, 500, 1000, and 2000 ms. Each pair of pulses was separated by 1 sec when the IPI was less than 500 msec and by 2 sec when the IPI was greater than or equal to 500 msec. The inter-block interval was 2 min. Pulses were delivered through a digital stimulator (Model DS8000, WPI Inc., Sarasota, FL, USA) and were rectified using a constant current stimulus isolation unit (A365; WPI Inc., Sarasota, FL, USA). The current range across experiments was 0.5 to 1.5 mA.

4.4. Taste stimuli and stimulus delivery

Taste stimuli consisted of 0.1 M NaCl, 0.01 M HCl, 0.01 M quinine and 0.5 M sucrose. Tastant concentrations were chosen so as to elicit half-maximal potentials in the CT nerve of the rat (Formaker et al., 1997). All taste stimuli were made from reagent-grade chemicals dissolved in distilled water and were delivered at room temperature. Stimulus presentation was accomplished through a custom built taste delivery system described in detail elsewhere (Di Lorenzo and Victor, 2003). Briefly, the system consisted of stimulus reservoirs pressurized with compressed air and connected via polyethylene tubing to a bundle of six stainless steel tubes placed in the mouth. Delivery of a tastant was controlled by computer activation of a solenoid valve interposed between the reservoir and the tongue. Flow rate was 5 ml/s. The taste solution bathed the whole mouth including the rostral 2/3 of the tongue, the field innervated by the CT nerve. This was verified by application of methylene blue through the system. Each stimulus trial consisted of 10 sec baseline, 10 sec of distilled water, 5 sec of tastant, 5 sec pause and 20 sec of a distilled water rinse. The inter-trial interval was 2 min.

4.5. Analysis of taste response properties

Electrophysiological responses to taste stimuli were obtained from single cell extracellular recordings. Signals were amplified with a Grass AC amplifier (Model P511, Astro-Med Inc., West Warwick, RI, USA). The magnitude of response to a given tastant was calculated as the mean firing rate (spikes per second; sps) during the 5 sec of tastant delivery minus the average firing rate (sps) during the 5 sec of water rinse that preceded tastant delivery. A taste-response was considered to be significant if the firing rate was 2.5 standard deviations greater than the firing rate during the last 5 sec of the preceding water rinse. Taste-responses were analyzed for breadth of tuning with an Uncertainty measure (Smith and Travers, 1979). The formula for Uncertainty was $H = -K \sum P_i (\log P_i)$, where K (scaling factor) = 1.66 and P_i was the proportion of response to stimulus i relative to the summed responses to all four stimuli. Values ranged from 0 to 1 with zero corresponding to a cell responsive to only one stimulus and 1.0 corresponding to a cell equally responsive to all four stimuli.

In addition to the Uncertainty measure, we calculated a metric called Selectivity that was designed to assess both the magnitude of response and the breadth of tuning. Selectivity was defined as the difference in response magnitude in sps between the sum of the two strongest responses and the sum of the two weakest responses. The formula for Selectivity was, $S = (t_1 + t_2) - (t_3 + t_4)$, where t_1 through t_4 indicate the response magnitudes to the first through the fourth best tastant. This measure is based on the assumption that the absolute magnitude of the response matters as much to the nervous system as the relative magnitude of response;

i.e. bigger responses are more important than smaller ones. Unlike the Uncertainty measure, which varies only between 0 (narrow tuning) and 1.0 (broad tuning), the Selectivity measure has no *a priori* upper bound. For example, two cells that each respond to a single stimulus might show Selectivity values of say 1 and 10 sps. This would indicate that the second cell shows a greater difference between its most and least effective stimuli compared with the first cell and might therefore convey a clearer signal about the identity of a tastant. The first cell would presumably convey little more information than a broadly tuned cell since the difference between its most and least effective stimuli is so small. Conversely, because the Uncertainty measure is based on proportional responsivity, these two cells might appear equally broadly or narrowly tuned.

4.6. Analysis of paired-pulse electrical stimulation

Paired-pulse stimulation data was assessed for the nature, duration and peak time of the paired-pulse effect. The occurrence of spikes following the conditioning and test pulses was used to construct a peristimulus time histogram (PSTH) of the evoked responses for about 100 trials at each IPI. The time at which the peak, earliest and latest evoked responses occurred in each PSTH, as well as the total number of evoked spikes were noted. The difference between the time of occurrence of the first evoked spike and the last was defined as "latency variability." These measures were used in a similar study of paired pulse stimulation of NTS cells in the caudal NTS (Scheuer et al., 1996). For each IPI the percentage change in the number of spikes following the conditioning and test pulses was calculated. A percentage change was used for comparisons across cells because of the variability in the total number of evoked spikes. This variability could be attributed to the proximity of the CT stimulating electrode to the nerve and the applied current level (range = 0.5 to 1.5 mA). Paired-pulse attenuation was defined as a decrease in the number of spikes following the test pulse compared to the number of spikes following the conditioning pulse, expressed as a percentage. Paired-pulse enhancement was defined as an increase in the number of spikes following the test pulse compared to the conditioning pulse, also expressed as a percentage. Significant paired-pulse effects were defined as greater than 25% change between conditioning and test pulse responses. The decay time constant was defined as the period between the onset of the conditioning pulse and the time at which the percent change reached 37% of maximum attenuation.

4.7. Histology

After recording taste and paired-pulse stimulation data from a cell, an electrolytic lesion was produced at the site of recording by passing DC current (1 mA cathodal; 5 sec) through the recording electrode. The animals were sacrificed with an overdose of pentobarbital and their brains were removed and placed in formalin. After 2 weeks the brains were frozen and the brainstem was sliced into 40 μ m sections, processed with cresyl violet, mounted on gelatinized slides and examined to verify the location of the lesion in the rostral NTS.

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Figure 1. Frequency distribution of latencies of evoked response to electrical stimulation of the CT nerve in all NTS cells recorded (n = 51). Black bars indicate taste-responsive cells; gray bars indicate non-taste-responsive cells.

Figure 2.

Effects of paired pulse stimulation of the CT nerve in one cell. A. Paired-pulse enhancement at an interpulse interval of 30 ms. More spikes occurred in response to the test pulse compared with the conditioning pulse. B. Paired-pulse attenuation at an interpulse interval of 50 ms. Fewer spikes occurred in response to the test pulse compared with the conditioning pulse. Each panel shows oscilloscope tracings of 100 sweeps superimposed.

Figure 3.

Frequency distribution of peak time of paired-pulse attenuation. Peak attenuation is defined as the interpulse interval at which the greatest percent decrement in the number of evoked pulses occurred between the conditioning and test pulses. Overall there was a bimodal distribution, especially for taste-responsive cells; however, for non-taste-responsive cells, the distribution was skewed toward longer interpulse intervals. Black bars indicate taste-responsive cells; gray bars indicate non-taste-responsive cells.

Figure 4.

Time course of paired pulse attenuation in NTS cells that showed early and late peak attenuation. Shown is the percent difference ($\pm$ SEM) in the number of evoked spikes in response to CT nerve stimulation following the conditioning pulse compared with the test pulse at various interpulse intervals. Cells with early peak attenuation show short lived time-dependent inhibition but cells with later peak attenuation show a gradual decline in time-dependent inhibition. Black bars show cells with early peak attenuation (≤ 20 ms); gray bars show cells with late peak attenuation (≥ 30 ms).

Figure 5.

Distribution of “best taste stimuli” among cells that showed early and late peak attenuation. Cells with early peak attenuation showed the greatest paired pulse attenuation at IPIs ≤ 20 ms and are indicated with black bars. Cells with late peak attenuation showed the greatest paired pulse attenuation at IPIs ≥ 30 ms and are indicated by gray bars. The best taste stimulus was defined as the tastant that evoked the largest response among those tastants tested.

Figure 6.

Location of cells recorded from the rostral NTS. A. Drawing of coronal sections of the brainstem showing the sites of electrolytic lesions indicating the location of NTS cells from which data were collected. Symbols are: star, taste-responsive cells; triangle, taste-responsive and non-taste-responsive cells recorded from approximately the same location; circle, non-taste-responsive cells. Numbers on upper right of drawing of each section indicate distance caudal to bregma. Abbreviations are: NTS, nucleus of the solitary tract; SpV, spinal nucleus of the trigeminal nerve. B. Photomicrographs of rostral NTS lesions showing the location of one NTS cell. Dotted line surrounds the NTS. Bars on lower right of each panel indicate 1.0 mm.