INTRODUCTION

Recent investigations have suggested that inhibitory interactions play a significant role in the neural processing of taste information in the brain stem. For example, Smith and Li (1998) have shown that almost two-thirds of the taste-responsive cells in the hamster nucleus of the solitary tract (NTS), the first central synaptic relay for gustatory information (Hamilton and Norgren 1984), are inhibited by GABA or released from inhibition by the GABA_A antagonist bicuculline methiodide (BMI) in a dose-dependent manner. Moreover, taste-driven neurons in the hamster NTS are subjected to tonic inhibitory influence (Smith and Li 1998; Smith et al. 1998). The experimental manipulation of this influence has been shown to directly alter the tuning profiles of single NTS neurons. Microinjection of BMI into the hamster NTS was shown to increase the breadth of responsiveness of NTS cells across taste qualities (Smith and Li 1998). These data suggest a prominent influence of inhibition on taste information processing in the NTS, although it is not understood how this inhibitory circuitry is normally activated and/or modulated.

Biophysical studies of rat NTS cells in vitro suggest that afferent sensory input normally activates inhibitory neural processes within the NTS (Bradley and Grabauskas 1998; Grabauskas and Bradley 1999). These studies demonstrated that tetanic electrical stimulation of the rostral NTS, at frequencies designed to mimic the rate at which spike volleys normally arrive, produced both short- and long-term potentiation of NTS inhibitory synaptic activity. Specifically, tetanus increased inhibitory postsynaptic potential decay time several hundredfold when compared with single-shock stimulation. This effect was observed to last from 5 to 15 min and sometimes for over 1 h (Bradley and Grabauskas 1998). Additionally, this sustained inhibition was reversibly blocked following application of BMI (Grabauskas and Bradley 1999). In light of these data, it is possible to speculate that normal afferent input may activate inhibitory circuitry in a manner that contributes to the neural processing of gustatory information within the NTS.

To test this hypothesis, electrophysiological responses to taste stimuli were recorded from single neurons in the rat NTS in vivo following high-frequency electrical stimulation of the chorda tympani (CT), a branch of the facial nerve that innervates the taste buds on the rostral two-thirds of the tongue. Additionally, paired-pulse electrical stimulation of the CT was used to determine the nature and time course of the inhibition activated by input arriving at the NTS via this nerve. If CT stimulation produced recurrent inhibition, then the NTS field response evoked by the second of two identical, temporally
proximal inputs should be of lesser amplitude than that evoked by the first, assuming that the second input arrives while the inhibitory activity remains effective. This type of paradigm has been used to study granule cell mediated inhibition of mitral/tufted cells in the olfactory bulb (e.g., Mori 1987).

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**METH O D S**

**Subjects**

Forty-three adult male Sprague-Dawley rats (350–475 g) were used as subjects. All animals were housed individually in stainless steel cages and maintained on a 12-h light-dark schedule; lights were turned on at 7:00 A.M. Temperature was maintained at 72°F. Food and water were available ad libitum.

**Surgery**

Prior to surgery, all rats were deeply anesthetized with urethane (1.5 g/kg, ip). Each animal was tracheotomized to facilitate breathing. Subjects were mounted in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA) with the incisor bar positioned 5 mm below the interaural line; this orientation allowed vertical access to the NTS without perturbing any major blood vessels or sinuses. The occipital bone was removed and parts of the cerebellum were gently aspirated to expose the Obex. The head was then fastened to a nontraumatic holder: stainless steel bone screws were embedded in the skull and cemented to a removable, ancillary arm of the stereotaxic device. This holder allowed for removal of the ear and incisor bars while the stereotaxic orientation of the head was retained. Body temperature was maintained at 37°C by a heating pad.

To prepare each animal for electrical stimulation of the CT, the pinna of the left ear was removed. A small amount of muscle and connective tissue was then excised to completely expose the lateral faces of the hard and soft palate. The entire surface of the tongue, including the trenches of the foliate papillae, was stained blue following these tests. Stained areas included part of the circumvallate papilla, the nasoincisor ducts, and the sur-}

**Electrophysiological recording in the NTS**

Etched tungsten microelectrodes, insulated except for the tip (18–20 MΩ at 1 kHz; FHC Inc., Bowdoinham, ME), were used to extracellularly record action potentials from single NTS neurons. The taste responsive portion of the NTS was located approximately 2.7 mm rostral and 1.8 mm lateral to the Obex and 1.0 mm ventral to the surface of the brain stem; only the NTS ipsilateral to the stimulated CT was studied. Action potentials recorded from single units were identified based on waveform consistency, which was continuously monitored throughout each recording session. Trains of action potentials that arose during experimentation were amplified (Grass PS11), pulse-code modulated, and stored, along with voice and trial marker cues, on VHS tape for off-line analysis.

**Tetanic electrical stimuli**

Electrophysiological responses to taste stimuli were recorded extracellularly from isolated, single NTS neurons both individually (i.e., control trials: normal taste stimulus presentation) and immediately following tetanic electrical stimulation of the CT (i.e., experimental trials: tetanus followed by taste stimulus presentation). Electrical pulse trains delivered to the CT consisted of 0.1-ms rectangular pulses (Grass S88 stimulator) that were rectified using a constant current stimulus isolation unit (Grass PSIU). The amount of current necessary to stimulate the CT and evoke neural activity in the NTS varied from subject to subject, most likely as a function of electrode placement relative to the nerve (Yeomans 1990). Therefore single pulse current density was adjusted on-line for each subject and set at a level slightly above the threshold needed to evoke NTS activity, which ranged from 0.5 to 1.5 mA.

In deciding on the frequency of tetanic stimulation to be used in this study, several factors were considered. Our intention was to mimic the frequency at which afferent impulses arrive at NTS via the CT.Recordings from single CT fibers have revealed taste-evoked response frequencies that ranged from 1 to 60 Hz (Ganchrow and Erickson 1970; Ogawa et al. 1968). Moreover, afferent firing frequency dynamically changes over the time course of a taste response, generally peaking in the first second (Ogawa et al. 1974), and is influenced by stimulus concentration (Ganchrow and Erickson 1970; Ogawa et al. 1974). Because many NTS cells are known to receive convergent input from more than one taste-related peripheral nerve (Grabauskas and Bradley 1996; Travers and Norgren 1995), the frequency of the combined incoming signals might be higher than the firing frequency of any single fiber (see Doetsch and Erickson 1970). Finally, it can be argued that the most meaningful signal for the nervous system is carried by the fibers that respond with the greatest frequency (see Gill and Erickson 1985). Given all of these factors, tetanic trains delivered at 50 Hz for 2 s were used in the present study to emulate a robust afferent volley from the CT to cells in the NTS.

**Taste stimuli**

Taste stimuli were chosen as prototypical representatives of the four “basic” taste qualities, i.e., sweet, salty, sour, and bitter: sucrose (500 mM), NaCl (100 mM), HCl (10 mM), and quinine-HCl (10 mM). Each was made from reagent-grade chemical stock dissolved in distilled water. The concentrations chosen for each stimulus evoked half-maximal responding from the rat CT (Ganchrow and Erickson 1970; Ogawa et al. 1974). Room temperature stimuli were presented to the subject’s oral cavity using a custom-built stimulus delivery system. Stimuli were stored in pressurized (∼10 lbs/in 2) reservoirs that were individually connected, via polyethylene tubing, to independent 1-mm-diam stainless steel tubes, each drilled with a series of small holes located along the entire length of the portion of the tube positioned in the rat’s mouth. These holes were located on opposite sides of each tube so that fluid could be directed toward the tongue and palate simultaneously. For each tube, an independent solenoid valve regulated fluid delivery; activation of this control valve flooded the entire mouth with tantast all at once. The solenoids were computer actuated, which allowed for precise control over stimulus presentation time. The flow rate of this system was approximately 5 ml/s. Although each tube was located in a slightly different position within the mouth, the high flow rate, along with the large number and position of the fluid delivery holes, ensured that each stimulus reached the entire mouth almost instantly.

Tests using methylene blue instead of a taste stimulus were performed on several rats to verify that the method of taste stimulus delivery adequately stimulated the receptive fields of the anterior and posterior oral cavities and the nasonocisor ducts. The location of stain within the oral cavity was examined with a dissecting microscope. The entire surface of the tongue, including the trenches of the foliate papillae, was stained blue following these tests. Stained areas included part of the circumvallate papilla, the nasonocisor ducts, and the surfaces of the hard and soft palate.
Tetanic-taste stimulation protocol

At the beginning of a data recording session, each taste stimulus was presented individually, and responses were recorded using the following parameters: 10-s baseline (i.e., spontaneous activity), 10-s stimulus presentation, 10-s pause, and a 20-s distilled water rinse. The inter-trial interval was ≥2 min. Next, taste responses were recorded following tetanic electrical stimulation of the CT. Initially, 10 s of spontaneous activity was recorded. A tetanic pulse train was then delivered to the CT for 2 s. At 1 s post-cessation of the electrical stimulus, a single taste stimulus was presented for 5 s, followed by a 5-s pause, and finally a 20-s distilled water rinse. This procedure was repeated for each taste stimulus using a 2-min inter-trial interval. When possible, trials that utilized 50-ms tetanus-taste stimulus intervals (TTI) were also recorded. Finally, each taste stimulus was individually presented again as per initial conditions. This protocol was then repeated as many times as possible for each isolated single unit to assess the within-neuron reliability of any tetanus-induced effect.

Data analysis: effects of tetanic stimulation of the CT on taste responses in the NTS

Action potentials arising from single units were converted to time-stamped transistor/transistor logic (TTL; sampling rate = 32 kHz) using the Discovery data acquisition package from DataWave Technologies (Longmont, CO). Single units were isolated in the following manner. Initially, an amplitude discriminator was used to screen waveforms that exceeded the background activity level. Next, for each waveform, eight metrics (e.g., spike amplitude; spike width; spike valley duration) were measured, and the results were plotted in a metric versus metric fashion. Action potential waveforms that arose from a single unit showed uniform shape and amplitude. Hence, they produced points in these plots that were grouped close together; the area within each plot that contained a cluster of these points was considered inclusive to a single unit. Any subsequent waveforms that produced points within this area were considered to have arisen from this same neuron. Each action potential was stamped with its time of occurrence (resolution = 1 ms) relative to the beginning of each stimulus trial.

For each unit, the latency of evoked spikes relative to the electrical pulses delivered to the CT nerve was calculated from tracings obtained from a storage oscilloscope. Because there was considerable jitter in the latency of evoked spikes in many cases, the latency of the evoked response was defined by the timing of the first spike in the group of evoked spikes for a given unit.

For each trial, mean spontaneous discharge rate was calculated as the average number of action potentials that occurred [i.e., spikes per second (SPS)] during the first 10 s of the trial. Due to the rapid response time of the stimulus delivery system (<100 ms from TTL “stimulus on” signal to washing of oral cavity with solution), taste response onset was defined as the time at which the first action potential arose during the first second of the taste stimulus presentation period. If a single spike did not appear during this time, taste response onset was defined as taste stimulus onset time plus a correction factor of 100 ms, which compensated for the slight lag noted above. The response magnitude evoked by each taste stimulus was defined as the average number of action potentials that occurred each second during the first 5 s of the taste response minus the mean spontaneous discharge rate measured during the 10-s baseline period. The stimulus response was considered to be significant if the magnitude was 2.5 SDs greater or less than the mean spontaneous discharge rate. No significant decreases in activity were recorded in response to any tastant either without or following tetanus.

Within each unit, the effect of tetanus was assessed and described by determining raw differences between initial and posttetanus responses to the same stimulus. A significant, tetanus-induced change in responding was defined as a change, either increase or decrease, of more than 50% of the initial response. For units with response rates of <5 SPS, changes of more than 100% or the appearance or complete disappearance of a response were required. Stricter criteria were necessary for these weak responses to rule out possible effects due to biological variation. In some cases changes due to tetanus were verified or ruled out by examination of replications. For stimulus trials that were recorded more than once from the same neuron, response magnitudes in each condition were averaged prior to determining the proportional effect of tetanus. To statistically evaluate the effect of tetanus within neurons from which ≥2 replications of responses to each stimulus were recorded under normal conditions and following tetanus, average initial and posttetanus responses to each stimulus were compared using dependent measures t-tests.

The effect of tetanus on taste responses as observed across our neuronal sample was statistically evaluated by using an appropriate ANOVA. For each ANOVA, degrees of freedom and P values for within-subject tests were corrected using the Greenhouse-Geisser adjustment to protect against violations of sphericity. Although these corrections were made prior to establishing P levels, only the corrected degrees of freedom and P values are reported. Posthoc comparisons among within-subject level means were accomplished through the use of dependent measure t-tests in which each observed score was evaluated using a Dunn critical value. This sort of multiple comparison procedure (MCP) is the only sort of posthoc test for repeated level means that has adequate control of alpha for all pairwise comparisons (Toothaker 1991).

The neuronal groupings suggested by cluster analysis were used in conjunction with an ANOVA to determine if tetanic electrical activation of the CT differentially affected neurons with different tuning profiles. Cluster analysis was performed to identify those units with tuning profiles most similar to each other, which therefore might be considered part of a functional group or type (see Smith et al. 1983). This analysis proceeded in a stepwise fashion: initially, the two neurons with most similar initial tuning profiles, as determined by Pearson product-moment correlation coefficients, were linked together to form a cluster. The next pair of most similar neurons, or the singular neuron most similar to the newly formed cluster, was then joined. As this process continued, new clusters were formed and/or new members were added to existing clusters until all elements were linked together. The degree of similarity between different clusters was expressed by the linkage distance between them within a dendrogram: dissimilarity (i.e., in this case low correlation) was denoted by long distances and a high degree of similarity (i.e., high correlation) was denoted by proportionately short distances. Distinct groups of units were then deduced from this solution. The Statistica (StatSoft, Tulsa, OK) cluster analysis module was used for the analysis; the Ward minimum variance linkage algorithm was applied.

To determine whether tetanic stimulation of the CT could affect the temporal organization of taste-evoked spike trains, the variability among interspike intervals within a taste response was examined before and after tetanic stimulation. Variability was measured as the SD of a sliding window of 10 successive interspike intervals. If spikes in a given sequence are regularly spaced in time, the SD of the interspike intervals in that train will be small. However, if the regularity of the spike train is disrupted, as might occur when there is a change in spike rate, then the SD will increase. These increases can signal the time points in the response when the spike rate is highly changeable. Only those cells that showed responses of more than 20 SPS and where there were at least two replications of taste stimulus presentations that were immediately preceded by tetanus were examined. In addition, units that showed significant changes in response magnitude following tetanus were excluded.

To describe the breadth of tuning of NTS taste cells, an Uncertainty measure (Shannon and Weaver 1949; Smith and Travers 1979) was calculated for each unit. The formula for Uncertainty was as follows.
where $P_i$ represents the response to each stimulus expressed as a proportion of the total response to $n$ stimuli and $K$ is a scaling constant. For four stimuli, $K = 1.661$, which results in $H$ ranging from a minimum of 0 (unit responds to only 1 stimulus) to a maximum of 1 (unit responds equally well to all stimuli). Because the formula for $H$ contains a log function, negative values for taste responses could not be used; those cells that contained negative responses were excluded from this analysis.

To determine whether there were differences in the effects of tetanic stimulation on taste responses between the two different TTIs, a stimulus (4 levels) by TTI (2 levels) repeated-measures ANOVA was performed. For every unit and each trial, responses were averaged for those recorded more than once and posttetanus responses were expressed as a percentage change from the respective initial response. These corrected scores were used as input to this ANOVA to allow for meaningful comparisons across neurons with different firing rates. Note that only 30 units were subjected to both TTIs and could be included in this analysis. The interaction between interval and taste stimulus was found to be nonsignificant. Additionally, no significant main effect of interval was noted ($\alpha = 0.01$). This suggested that, across this sample, the average effect of tetanus on taste responding was the same at both 1,000- and 50-ms TTIs. Therefore for all subsequent across-unit analyses, posttetanus responses were collapsed across TTI.

**Field potential recording protocol**

Field responses, each evoked by single-shock stimulation of the CT, were recorded from the gustatory portion of the NTS with the same microelectrodes as in the tetanic stimulation protocol. Paired, singular electrical pulses were generated with a Grass S88 stimulator. Each rectangular pulse (0.1 ms) was rectified using a constant current stimulus isolation unit (Grass, PSIU). The interval between the initial “conditioning” and secondary “test” pulse was systematically varied. The following conditioning-test intervals were used: 10, 20, 30, 50, 100, 500, 1,000, and 2,000 ms. For each preparation, a block of $\geq 10$ conditioning-test trials was generally recorded for each interval, using a 10-s inter-trial interval. As with tetanic stimulation, the amount of current necessary to stimulate the CT and evoke a field potential was pulse-code modulated from DataWave Technologies. For each trial, the amplitude of the field response was analyzed using the Evoked Potential module of c Workstation, a neural data analysis software package from DataWave Technologies. For each trial, the amplitude of the field response evoked by the test pulse was compared with that evoked by the conditioning pulse. In the olfactory system, attenuation of test pulse field response amplitude has been shown to be directly due to inhibition activated by the conditioning pulse (Mori 1987). Field response amplitude data were normalized to allow comparisons across different preparations with respect to the relative, rather than absolute, magnitude of response. For each pair, test pulse field response amplitude was expressed as a proportion of conditioning pulse field response amplitude.

**Data analysis: field potentials evoked by paired pulse electrical stimulation of the CT**

Each field potential was converted to digital form (sampling rate = 26 kHz) using the Discovery data acquisition package. Digital field response data were analyzed using the Evoked Potential module of Personal Scientific Workstation, a neural data analysis software package from DataWave Technologies. For each trial, the amplitude of the field response evoked by the test pulse was compared with that evoked by the conditioning pulse. In the olfactory system, attenuation of test pulse field response amplitude has been shown to be directly due to inhibition activated by the conditioning pulse (Mori 1987). Field response amplitude data were normalized to allow comparisons across different preparations with respect to the relative, rather than absolute, magnitude of response. For each pair, test pulse field response amplitude was expressed as a proportion of conditioning pulse field response amplitude.

**RESULTS**

Electrophysiological responses to taste stimuli presented both individually and following tetanus were recorded from 46 NTS units. The mean spontaneous firing rate observed across all units prior to tetanus was $2.23 \pm 0.44$ (SE) spikes/s. Posttetanus taste responses to each stimulus were recorded from all cells using a 1,000-ms TTI; in 30 units, both 1,000- and 50-ms TTIs were tested. Pairs of evoked NTS field responses were recorded from seven preparations.

Units were classified according to their “best” stimulus, defined as the tastant that evoked the largest response. Twenty-seven (58%) of the 46 units responded best to NaCl (N best), 9 (20%) responded best to HCl (H best), 9 (20%) responded best to sucrose (S best), and 1 (2%) responded best to quinine (Q best).

The mean response rates to all stimuli before and after tetanus are shown in Table 1. The order of stimulus effectiveness without prior tetanic stimulation was NaCl > HCl > sucrose > quinine. Generally, NTS units were broadly responsive across taste stimuli: the average Uncertainty measure was 0.69 ± 0.03. Prior to tetanus, 18 (39%) units significantly responded to all taste stimuli, 12 (26%) significantly responded to three stimuli, 7 (15%) responded to two stimuli, and 9 (20%) significantly responded to only one taste stimulus.

**Evoked responses to electrical stimulation of the CT nerve**

Evoked responses to electrical stimulation of the CT nerve were analyzed in 38 taste-responsive units by examination of oscilloscope tracings. In the remaining eight cells of the sample, the evoked responses were difficult to analyze due to the presence of both taste responsive and unresponsive units with evoked responses; these units were excluded from this analysis. Six of the 38 units examined showed no evoked response to CT electrical stimulation. These included three units that were narrowly tuned to sucrose, two units that were N best but which also had a strong response to sucrose, and one unit that was narrowly tuned to NaCl.

Electrical stimulation of the CT often evoked a clear com-

**TABLE 1. Mean response rates evoked by taste stimuli across all experimental conditions**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Mean</th>
<th>SE</th>
<th>n Trials</th>
<th>Significant Responses (% Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>without tetanus (n = 46)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>7.1</td>
<td>1.0</td>
<td>129</td>
<td>70 (54)</td>
</tr>
<tr>
<td>NaCl</td>
<td>22.8</td>
<td>3.0</td>
<td>123</td>
<td>111 (90)</td>
</tr>
<tr>
<td>HCl</td>
<td>13.6</td>
<td>2.3</td>
<td>120</td>
<td>86 (72)</td>
</tr>
<tr>
<td>Quinine</td>
<td>6.4</td>
<td>1.3</td>
<td>122</td>
<td>67 (55)</td>
</tr>
<tr>
<td>following tetanus (1,000-ms tetanustastant interval; n = 46)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>5.8</td>
<td>1.0</td>
<td>100</td>
<td>50 (50)</td>
</tr>
<tr>
<td>NaCl</td>
<td>21.4</td>
<td>3.0</td>
<td>95</td>
<td>88 (93)</td>
</tr>
<tr>
<td>HCl</td>
<td>11.3</td>
<td>1.9</td>
<td>93</td>
<td>57 (61)</td>
</tr>
<tr>
<td>Quinine</td>
<td>4.7</td>
<td>1.0</td>
<td>99</td>
<td>48 (48)</td>
</tr>
<tr>
<td>following tetanus (50-ms tetanustastant interval; n = 30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>6.3</td>
<td>1.3</td>
<td>73</td>
<td>40 (55)</td>
</tr>
<tr>
<td>NaCl</td>
<td>21.1</td>
<td>3.3</td>
<td>72</td>
<td>67 (93)</td>
</tr>
<tr>
<td>HCl</td>
<td>9.3</td>
<td>2.1</td>
<td>67</td>
<td>38 (57)</td>
</tr>
<tr>
<td>Quinine</td>
<td>4.1</td>
<td>0.9</td>
<td>72</td>
<td>36 (50)</td>
</tr>
</tbody>
</table>
pound action potential, which lasted approximately 3 ms and was comprised of four distinct components. The amplitude of each of these components varied from preparation to preparation. Figure 1 (top) shows an example of this potential. Neither the presence/absence nor the latency of the evoked response in a given unit was correlated with the presence or amplitude of the compound action potential.

One or two spikes were observed following each pulse of the tetanic train in the 33 units that showed evoked responses to CT stimulation. Mean response latency across all cells with evoked responses was $3.8 \pm 0.1$ ms. The distribution of the latencies of the evoked responses is shown in Fig. 2. In all cases, units did not produce a spike following every electrical pulse, although the degree of following appeared to relate to the latency of the evoked response. That is, neurons that exhibited short-latency responses were generally better followers and showed less variable response latency than those cells with longer response delays (see Fig. 1). In six units with evoked response latencies of 3 ms, CT stimulation often evoked a second spike at approximately 2 ms following the first. In general, units with the largest responses to NaCl showed the shortest latencies and were relatively narrowly tuned; units that displayed longer latencies were generally more broadly tuned and showed smaller responses across all tastants. Figure 3, which illustrates this point, shows the response profiles of the five units that showed the shortest latency and the five units that showed the longest latencies of response to CT stimulation.

**Effects of tetanus: within unit analysis**

Of the 46 taste-responsive NTS units that were tested before and after tetanic stimulation of the CT, 18 (39%) met the criteria for a significant change in at least one taste stimulus response following tetanic CT stimulation. The response to a single stimulus was changed in 13 units and responses to two stimuli were changed in 5 units. For sucrose responses, there were five significant decreases in response recorded at the 1-s TTI. In one unit, there was a significant response to sucrose following tetanus where no response to this stimulus was initially observed. Three sucrose responses were significantly attenuated following tetanus at the 50-ms TTI; all of these units also showed attenuated sucrose responses at the 1-s TTI. For

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**FIG. 1.** Oscilloscope tracings (50 overlays) of the following. A: compound action potential following tetanic stimulation of the chorda tympani nerve (CT) in a cell without an evoked response. Each of 4 components is labeled with a number. B: compound action potential and evoked response in an NTS unit with an evoked response at approximately 4 ms following tetanic stimulation of the CT. C: compound action potential and evoked response in an NTS unit with an evoked response at approximately 3 ms following tetanic stimulation of the CT. Solid line indicates 1 ms.

**FIG. 2.** Distribution of response latencies across NTS units evoked by tetanic stimulation of the CT.

**FIG. 3.** Response profiles of the 5 units with the shortest latencies of response to tetanic CT stimulation (■) and the 5 units with the longest evoked response latencies (□). S, sucrose; N, NaCl; H, HCl; Q, quinine.
NaCl responses, only one significant decrease was recorded at the 1-s TTI (the 50-ms TTI was not recorded) and one significant increase was recorded at the 50-ms TTI, but not at the 1-s TTI in the same unit. For HCl, there were six significantly attenuated responses and two significantly augmented responses at the 1-s TTI. One of these units showed a response to HCl following tetanus where none was present without prior tetanic stimulation. At the 50-ms TTI, one significant decrease and one significant increase in responding to HCl were recorded following tetanus. Two units also tested at the 50-ms TTI also showed a significant decrease in their response to quinine following tetanus.

An analysis was performed to ascertain whether tetanic stimulation had a greater effect on responses to the least effective stimulus compared with responses to the most effective stimulus. Taste responses within all cells that were affected by tetanus were ranked according to response magnitude. The rank of the stimulus that was affected was then noted. Results showed that seven responses affected by tetanus were evoked by the second most effective stimulus for a given cell. Eight responses affected by tetanus were evoked by the third most effective stimulus and eight responses affected by tetanus were evoked by the least effective stimulus within a given cell.

Further analyses were conducted on neurons from which at least two recordings of responses to each stimulus were obtained both before and after tetanus at either interval. Twenty-four neurons met this criterion. For this sample, normal responses to each stimulus were recorded 3.8 times on average per neuron; responses to stimuli presented 1,000 and 50 ms following tetanus were recorded 3.2 and 3.4 times on average per unit, respectively. Taste responses measured individually (i.e., not preceded by tetanus) in these units were found to be stable across repetitions; there was no general decrease in responsiveness over the recording session. The median SE of the average response magnitudes across all stimuli that were presented at least three times \((n = 24)\) units was 0.89 SPS (range, 0.03–3.93 SPS). For those units where all tastsants were presented at least four times \((n = 13)\), the mean response rates \((\text{SPS})\) ± SE for presentations one to four were as follows: for NaCl, 31.8 ± 6.5, 33.1 ± 6.5, 30.0 ± 6.1, 31.3 ± 6.2, respectively; for HCl, 14.0 ± 4.6, 14.9 ± 4.3, 15.5 ± 4.8, 15.2 ± 5.1, respectively; for quinine, 5.2 ± 2.4, 5.6 ± 2.0, 6.0 ± 2.3, 6.4 ± 2.2, respectively; for sucrose, 6.2 ± 1.9, 6.6 ± 2.1, 5.1 ± 2.3, 5.2 ± 2.1, respectively.

Within this sample of 24 neurons, tetanic electrical activation of the CT significantly altered taste responses in seven of the units examined (dependent measures \(t\)-tests, \(\alpha = 0.05\)). The proportion of units with replications that showed effects of tetanus (29%) was slightly less than the proportion of all units that showed effects of tetanus (39%). Twenty-six significant changes in taste response magnitude were found following tetanic stimulation. Thirteen of these changes also represented

![Graphs of taste responses to NaCl, HCl, sucrose, and quinine before and after tetanic stimulation.](https://example.com/graph4.png)

**FIG. 4.** Responses of 46 taste-responsive NTS neurons to 100 mM NaCl, 10 mM HCl, 10 mM quinine, and 500 mM sucrose without tetanus (without) and following tetanic stimulation of the CT (following tetanus). When stimuli were presented more than once, response rates were averaged. \(\square\) responses where a 1-s tetanus-taste stimulus interval (TTI) was used; \(\bullet\) responses where a 50-ms TTI was used. Dotted line in each graph indicates when “without” after tetanus.” Solid line in each graph and the equation at the top of each graph represent the result of a linear regression analysis fitted to the data.
a >50% change from baseline. Each change was response attenuation with one exception: the response to HCl was significantly enhanced following tetanus in one unit. Differential effects of long and short TTIs were noted at the single-neuron level: 8 of 96 (8%) taste responses were significantly attenuated at the 50-ms but not the 1,000-ms TTI.

An example of the test-retest reliability of the effects of tetanic stimulation of the CT on NTS taste responses is shown in Fig. 5. This graph shows the average responses to quinine in four units over three sequential replications of the tetanic stimulation. It can be seen that all quinine presentations that were preceded by tetanic stimulation of the CT nerve were attenuated with respect to those that were presented without tetanic stimulation.

To illustrate the effects of tetanus on taste responses in one unit, the peristimulus-time histograms associated with pre- and posttetanus responses to each taste stimulus from a selected recording are shown in Fig. 6A. Initially, this unit showed robust responses to quinine (33.3 average SPS above spontaneous discharge, measured over 5 s) and NaCl (28.7 SPS), and lesser responses to HCl (19.0 SPS) and sucrose (7.3 SPS). Following tetanic electrical stimulation of the CT, the response to quinine was attenuated at the 1,000-ms TTI (19.3 SPS); further attenuation was noted if the TTI was shortened to 50 ms (3.0 SPS, nonsignificant response). Similar trends were noted for NaCl (1,000-ms TTI: 12.8 SPS; 50-ms TTI: 10.4 SPS), HCl (1,000-ms TTI: 6.2 SPS, nonsignificant response; 50-ms TTI: 2.5 SPS, nonsignificant response) and sucrose (1,000-ms TTI: −0.1 SPS, nonsignificant response; 50-ms TTI: −0.8 SPS, nonsignificant response). Figure 6, B and C, shows examples of raw recordings from this unit. On the left (B) are examples of quinine responses before and after tetanic CT stimulation and on the right (C) are oscilloscope tracings of the waveform.

**Effects of tetanus: temporal organization of taste responses**

If tetanic electrical stimulation of the CT evoked inhibitory activity in the NTS, it is possible that the manifestation of this influence might be more subtle than a simple attenuation of response magnitude. Since the great majority of taste responses in the NTS are excitatory, it is possible to argue that the excitation generated in taste afferents by a taste stimulus is a more potent determinant of NTS activity than any ancillary inhibitory influence. In this context, the suppressive effects of NTS inhibitory activity may not prevent spikes from occurring entirely but rather impose temporal order within trains of taste-evoked action potentials by delaying their occurrence. To investigate this possibility, the variability among interspike intervals within a taste response was measured before and after tetanic stimulation. Fifteen units met the criteria for inclusion in this analysis, as defined in the method section. Among these, there were 2 sucrose responses, 14 NaCl responses, 9 HCl responses, and 2 quinine responses that were >20 SPS above baseline. An increase in the variability of the interspike intervals following tetanus was found in seven units: in each affected unit, this phenomenon was only observed in the response to one tastant even though each unit commonly responded to more than one taste stimulus. Four of these seven units showed changes in response magnitude to a different stimulus in addition to the noted changes in the variability of

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**FIG. 5.** Mean response rates (±SE) for sequential presentations of quinine in 4 units. Stimulus presentations without prior CT stimulation (Q1–Q4) alternated with stimulus presentations that were preceded by CT stimulation (EQ1–EQ4).

**FIG. 6.** A: peristimulus-time histograms of responses to taste stimuli before and after tetanic stimulation of the CT. For each stimulus, the top histogram shows the response without tetanus; middle, 50-ms TTI; and bottom, 1,000-ms TTI. Bar below histograms shows the presentation of tetanus. Arrow indicates the onset of a 5-s taste stimulus. Absence of neural activity during tetanus is a result of filtering; spikes could not reliably recorded during the tetanic stimulus due to stimulus artifact. Time bin = 250 ms. B: raw responses to quinine in the same unit shown in A. Each trace shows 5 s of activity; solid line under each trace indicates stimulus presentation. Top, quinine without tetanus; middle, quinine with a 50-ms TTI; and bottom, quinine with a 1,000-ms TTI. C: oscilloscope tracings of waveform of the unit depicted in A. Panel shows approximately 20 sweeps superimposed. Solid line under trace indicates 1 ms.
the interspike intervals. Four NaCl responses, two HCl responses, and one sucrose response showed more variability in the sequence of interspike intervals following tetanus. In each case, this effect occurred within the first 2 s of the response.

Figure 7 shows an example of this analysis applied to NaCl responses in NTS units E18 and E46. In Fig. 7A it can be seen that there are no differences in the interspike interval histograms of NaCl responses before \((n = 4)\) and after \((n = 3)\) tetanic stimulation of the CT in E18. Figure 7B shows that the peristimulus-time histograms of NaCl responses in this unit are indistinguishable. In Fig. 7C (top), the results of the analysis of variability of sequential interspike intervals are shown for E18. It can be seen that CT stimulation disrupted the temporal organization of the spike train in the initial portion of the response, i.e., the first 100 interspike intervals or approximately the first 2 s of response, but had no effect on the remaining portion of the response. In a second NTS cell, E46, (Fig. 7C, bottom) there was no effect of CT stimulation on the response to NaCl \((n = 4\) without CT stimulation, \(n = 4\) following CT stimulation). In this unit, the interspike intervals were longer than those in the NaCl responses for E18 and therefore might be expected to show larger variability. However, the first 2 s of response, where effects of tetanus were seen in E18, were completed by the 50th spike, and there was no comparable effect of tetanus on the variability of interspike intervals. (It can be seen in the figure that there were a few interspike intervals where the average SD appeared to be larger with tetanus than without. These interspike intervals occurred at approximately 1.5 s into the response and encompassed a period of <200 ms.)

Effects of tetanus: breadth of tuning

The effect of tetanic electrical stimulation of the CT on neuronal breadth of tuning was assessed by comparing \(H\) values determined both prior to and following tetanus. Forty-one neurons were included in this analysis. For stimulus trials that were recorded more than once from the same neuron, response magnitudes were averaged prior to calculating \(H\). The mean Uncertainty value for all units was \(0.70 \pm 0.03\) before tetanus and \(0.66 \pm 0.03\) following tetanus. These values were not significantly different. Of the 19 units that were affected by tetanus, the average Uncertainty values without tetanus was \(0.70 \pm 0.05\) and \(0.65 \pm 0.05\) following tetanus. Eight of these units showed an increase in Uncertainty following tetanus while 11 units displayed a decrease in Uncertainty after tetan-

![FIG. 7. A: interspike interval histogram showing the distribution of interspike intervals in unit E18 during NaCl responses without \((n = 4)\) and following tetanic stimulation \((n = 4)\) of the CT. B: peristimulus-time histograms of a single NaCl response without (top) and after (bottom) tetanic CT stimulation. Bar below histograms shows the presentation of tetanus. Arrow indicates the onset of a 5-s taste stimulus. C: plot of the SD of a sliding window of 10 sequential interspike intervals during the response to NaCl without and following tetanic stimulation of the CT in unit E18 (top: \(n = 4\) without and \(n = 3\) following tetanus) and E46 (bottom: \(n = 4\) without and following tetanus).]
nus. Figure 8 (top) shows the response profiles of four neurons where posttetanus $H$ values that were lower than those measured initially by (arbitrarily) more than 0.25. For each case, the change in tuning was almost fully accounted for by a suppression of responding to nonbest stimuli; the response to the best stimulus was preserved and/or sometimes slightly facilitated. A similar trend was noted in one additional neuron for which $H$ could not be calculated due to negative response magnitude values. There were no units that increased their Uncertainty by more than 0.25. However, Fig. 8 (bottom) shows the response profiles of the two neurons that showed the greatest increase in posttetanus $H$ values compared with those measured initially. It can be seen that the increase in the breadth of tuning in these units was due to a stimulus-selective increase in posttetanic response magnitude. Changes in Uncertainty for other units that became more broadly tuned following tetanic stimulation were all less than 0.09.

**Effects of tetanus: across unit analysis**

Hierarchical cluster analysis was used to categorize units into distinct groups based on similarities among their initial response profiles. The effect of tetanus on taste responses across all 46 neurons

**FIG. 8.** Response rate (SPS) for all taste stimuli without and following tetanic CT stimulation in 5 units that became more narrowly tuned following tetanus (top) and 2 units that became more broadly tuned following tetanus (bottom). $H_w$ denotes the Uncertainty measure of each unit based on taste responses without tetanic stimulation and $H_t$ denotes the Uncertainty measure of each unit based on taste responses following tetanic stimulation. In 1 unit (bottom left), the Uncertainty measure could not be calculated because of negative response rates.

**FIG. 9.** Results of hierarchical cluster analysis. Neurons are identified by their best stimulus (N, NaCl; H, HCl; Q, quinine; S, sucrose) and by an integer that represents the rank of the response magnitude in relation to other units in their category; i.e., N3 is an N best unit with third highest response to NaCl. Inset: graphs show the average response profile for each of 3 groups of units without (striped bars) and following (filled squares) tetanic CT stimulation. Positive-going error bars are associated with responses without tetanus and negative-going error bars are associated with responses following tetanus.
in the sample was statistically evaluated using a neuronal group (3 levels) by stimulus (4 levels) mixed ANOVA. Neuronal group was considered a factor to determine if neurons with similar tuning profiles reacted to tetanus in a similar way. For every unit and each trial, responses were averaged for those recorded more than once and posttetanus taste responses, collapsed across TTI, were expressed as a percentage change from their respective initial responses. These percentage change scores were used as input to the ANOVA to allow for meaningful comparisons across neurons with different firing rates. A significant main effect of stimulus was found \[F(3,129) = 4.36, P = 0.006\]. Posthoc exploration of this effect revealed that the mean quinine percentage change was significantly lesser (i.e., greater suppression) than that for NaCl (Dunn MCP, \[T_{45} = 3.82, P = 0.0004\]; see Fig. 10), which implied that tetanic stimulation of the CT attenuated quinine responses to a significantly larger extent than NaCl responses. Mean sucrose and HCl percentage change scores were also lesser than NaCl; however, these differences were not significant. The main effect of neuronal group and the group by stimulus interaction were not statistically significant, which suggested that the effect of tetanus was not restricted to a particular class of neuron.

**Population field potentials**

A total of 424 pairs of field responses were recorded from seven preparations in response to paired pulse single-shock stimulation of the CT (44 pairs were recorded at the 10-ms conditioning-test interval, 72 pairs at 20 ms, 41 pairs at 30 ms, 67 pairs at 50 ms, 65 pairs at 100 ms, 56 pairs at 500 ms, 47 pairs at 1,000 ms, and 31 pairs at 2,000 ms). For each recording preparation, average test field response amplitude, as measured from the absolute trough to absolute peak, was significantly attenuated for each conditioning-test interval up to and including 100 ms (dependent measures \(t\)-tests, \(\alpha = 0.05\)). With the exception of one preparation, average test field responses were also significantly suppressed at the 500-ms interval. Test field responses were significantly attenuated for one preparation at the 1,000-ms interval. No significant suppression of test response amplitude was observed at the 2,000-ms interval.

Figure 11 shows raw data from a single preparation. As shown, each evoked field response consisted of an initial negative-going potential followed by positive-going deflection, which was indicative of the hyperpolarization and subsequent depolarization of the extracellular space that occurs during neural activation. It may be seen that the field response evoked by the test input was dramatically suppressed when the interval between the conditioning and test inputs was 20 ms. However, negligible attenuation was noted at the 500-ms interval.

The relative amount of test response suppression varied as a function of the interval between the conditioning and test pulse. Figure 12 describes the degree of test response suppression observed at each interval across the entire data set. In this figure, test response amplitude is expressed as a percentage change of conditioning response amplitude. A linear relationship between the degree of test response suppression and conditioning-test pulse interval was found. Attenuation was largest at shortest intervals and lessened as the interval was lengthened. This linear relationship was found to be statistically significant.
DISCUSSION

Taste responses were recorded from 46 neurons in the NTS of anesthetized rats before and after tetanic electrical stimulation of the ipsilateral CT nerve. Electrical pulses delivered to the CT were found to produce a compound action potential with four components. Taste-driven units in the NTS showed evoked responses that varied in latency and strength. Those cells that showed strong, short latency responses to CT stimulation responded vigorously to NaCl and were relatively narrowly tuned. Units with longer latencies generally responded more broadly to taste stimuli and with lower response magnitudes. Following tetanus, taste responses in 21 (46%) of the 46 units were reversibly altered in a stimulus-selective manner.

Latency of evoked responses to CT stimulation was related to taste response magnitude in general and to the magnitude and specificity of responsiveness to NaCl in particular. This is perhaps not surprising considering that the CT nerve is known to contain a large proportion of NaCl-specific fibers (Frank et al. 1983). Assuming these afferents feed NTS neurons that respond strongly to NaCl, present results suggest that these are among the largest diameter and, hence, fastest conducting fibers in the CT since they evoke NTS responses of relatively short latency. In addition, the relationship between evoked response latency and breadth of tuning might correlate with patterns of convergent input from the CT and other taste-related afferents. This possibility has yet to be investigated.

Evoked responses to CT stimulation

The compound action potentials recorded in the NTS following electrical stimulation of the CT reflect the heterogeneity of fiber types in the CT nerve (Hanamori et al. 1996). The present finding of four distinct components to this potential, each presumably corresponding to volleys from fibers of different conduction velocities, agrees with results of a similar study of CT stimulation in anesthetized rats (Hanamori et al. 1996).

The latencies of evoked spikes following CT stimulation were generally shorter than those previously reported for gustatory NTS neurons (Ogawa and Kaisaku 1980, 1982). One explanation is that we were not able to detect cells with evoked response latencies longer than 20 ms because our stimulation was delivered at 50 pulses/s. This may also explain why some cells appeared to show no evoked response, although these cells may have received taste-related input from other nerves. Another possibility is that these relatively short latency responses (e.g., 3 ms) were actually antidromic. The observation that those units with the shortest latency evoked responses showed the least latency jitter and the strongest evoked responses, i.e., most if not all electrical pulses evoked a spike, supports this notion. To confirm this possibility, evoked responses following systematic variation in both amplitude and frequency of the electrical stimulation would need to be recorded. On other hand, the lack of any antidromic responses recorded in taste-responsive units in previous studies (Ogawa and Kaisaku 1980, 1982) argues against the idea that these short latency responses were due to antidromic activation. It is also possible that the use of barbiturate anesthesia in previous studies may have prolonged the latency of evoked responses in NTS units. Latencies of evoked responses following electrical stimulation of the solitary tract in vitro (mean latency = 4.8 ± 0.6 ms; Grabauskas and Bradley 1996) were shorter than those recorded under barbiturate anesthesia (7.2 ± 4.3; Ogawa and Kaisaku 1982) and were more comparable to those reported here. However, differences noted relative to the present study may also be attributed to the site of CT stimulation, because Ogawa and Kaisaku (1980, 1982) used a submandibular approach.

Gustatory afferents and inhibition

Grabauskas and Bradley (1998) have shown that local tetanic stimulation of NTS cells in vitro following blockade of excitatory neurotransmission potentiated inhibitory postsynaptic potentials for as long as 1 h following tetanus. They further demonstrated that this potentiation is mediated by GABA A receptors on NTS cells (Grabauskas and Bradley 1999). These authors suggested that their tetanic stimulation protocol might be analogous to a natural afferent volley that would be conveyed by a taste nerve, and further, that afferent input would invoke both excitatory and inhibitory activity in the NTS neuronal network. Results of the present study are generally consistent with this idea. When the average effect, as viewed across our sample of neurons, was considered, tetanic stimulation of the CT nerve produced stimulus-specific response attenuation, particularly affecting quinine responses. In addition, population field potential recordings following paired pulse stimulation of the CT nerve suggested that CT input to the NTS induces a period of attenuated responsiveness, consistent with an inhibitory influence. Although these effects may have resulted from depletion of neurotransmitter from presynaptic terminals, loading of the extracellular space with K + and/or postsynaptic desensitization, the observation that the effect of tetanus was stimulus-specific argues against these possibilities.

In light of evidence that inhibitory processes influence the breadth of tuning of taste responsive cells in the NTS (Smith and Li 1998), we expected that tetanic stimulation of the CT would affect the breadth of tuning of neurons from which we recorded. However, such changes were found only infrequently following tetanus, with both increases and decreases in uncertainty observed. Interestingly, the tetanus-induced sharpening of tuning that was noted for some neurons was accomplished
mainly through a selective reduction in the responses to non-best taste stimuli. These observed changes in breadth of tuning are consistent with the idea that tetanus enhanced inhibitory activity in the NTS.

Analyses of changes in the temporal organization of taste responses following tetanus suggest that the effects of tetanic stimulation of the CT may be more complex than a simple reduction of spike rate. Initially, these analyses demonstrated that taste-evoked spike trains may show a replicable temporal pattern, as others have suggested (Erickson et al. 1994; Katz et al. 2001). When the SD of sequential interspike intervals is low, it implies that successive interspike intervals are similar so that spikes occur at approximately regular intervals. Conversely, when the SD of sequential interspike intervals is high, this regularity in the firing pattern is absent. In this study, tetanic stimulation of the CT disrupted the regularity of interspike intervals (as demonstrated by an increase in the SD of sequential interspike intervals) over approximately the first 2 s of the response. One possible explanatory mechanism may be that these results reflect the biphasic inhibitory post synaptic potential (IPSP), i.e., an IPSP followed by a burst of spikes, observed in NTS cells in vitro following tetanic stimulation at 50 Hz (Grabauskas and Bradley 1998). In effect, these bursts of spikes induced by tetanus may intrude into the orderly progression of the taste-evoked spike train. Alternatively, by inducing long-lasting inhibitory potentials, tetanic stimulation may disrupt the ability of inhibitory interneurons to regulate the timing of response-related firing patterns.

Although Grabauskas and Bradley (1998) essentially demonstrated posttetanic potentiation of inhibitory potentials in every cell from which they recorded, tetanic stimulation of the CT in the present study only affected 43% of NTS units. It is possible that convergent input from the CT and other taste-related afferents might interact in such a way as to mask any inhibition produced by tetanic CT stimulation in vivo. Such interactions have been proposed in the literature (e.g., Grabauskas and Bradley 1996), although the evidence is controversial (see Dinkins and Travers 1998).

The selective attenuation of responses to quinine that was observed following tetanic activation of the CT might reflect a mutually inhibitory relationship between the CT and other taste nerves, as first suggested by Halpern and Nelson (1965). For example, attenuation of quinine responses in sucrose/quinine mixtures has been reported (Smith et al. 1994; Vogt and Smith 1993) in the hamster parabrachial pons. Smith et al. (1994) have argued that the quinine/sucrose antagonism observed both neurophysiologically in the brain stem and behaviorally (Berridge and Grill 1984) may reflect inhibitory interaction between the VIIth and IXth nerves, given that these afferents exhibit differential sensitivities to sweet and bitter stimuli (Frank 1991; Frank et al. 1983). However, in light of recent data (Dinkins and Travers 1998), inhibitory interactions among gustatory afferents arriving at the NTS are not fully understood.

Neural representation of taste quality and inhibition

Although tetanic stimulation of the CT was meant to serve as an analog to a natural afferent volley, it was an aphysiological manipulation. That is, unlike a steady train of pulses, a natural afferent volley of spikes would presumably be subject to ongoing modification by inhibitory processes that it generates through inhibitory interneurons. These modifications may represent the true effect of inhibition on neural responses. With tetanic stimulation, the effects of inhibitory processes produced by the pulse train would naturally interact with the effects of inhibitory processes produced by subsequent taste responses. The combination of the two processes might therefore attain a “ceiling” whereby no further inhibition is possible. This might account, at least partially, for the relatively infrequent effects that were observed in the present study. However, it would not account for the stimulus-selectivity of the effects of tetanic stimulation. This aspect of the effects of tetanus may provide clues as to the role of inhibition in neural taste information processing.

Assuming that afferent activity from the CT activates a recurrent inhibitory process within the NTS, the function of this inhibition in the neural code for taste may be to facilitate contrast in the neural representation of different taste qualities. Inhibition within the gustatory NTS could serve to fine tune across neuron patterns of response, which are believed to underlie the neural code for taste (Erickson 1963; Pfaffmann 1959; see also Scott and Giza 2000; Smith and St. John 1999). The strength of the evoked inhibition activated by individual neurons might vary as a function of stimulus quality; more robust responses would produce more inhibition than weak ones, as in the present study. Activation of the best stimulus of a neuron would naturally evoke the most intense inhibition. Inhibitory processes activated during the response to a given taste stimulus might attenuate the responses to that stimulus in neurons that would respond only weakly otherwise. This would have the effect of restricting the activity generated by the stimulus to those cells that are most sensitive to it, increasing contrast in across neuron patterns of response evoked by different taste stimuli. The alternative without inhibition would be a more broadly distributed pattern of sensitivity where every neuron would respond maximally to every stimulus (see Di Lorenzo 2000).

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