Amygdalofugal Influence on Processing of Taste Information in the Nucleus of the Solitary Tract of the Rat

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Kang Y, Lundy RF. Amygdalofugal influence on processing of taste information in the nucleus of the solitary tract of the rat. J Neurophysiol 104: 726–741, 2010. First published June 2, 2010; doi:10.1152/jn.00341.2010. Previous studies have shown that corticofugal input to the first central synapse of the ascending gustatory system, the nucleus of the solitary tract (NST), can alter the way taste information is processed. Activity in other forebrain structures, such as the central nucleus of the amygdala (CeA), similarly influence activation of NST taste cells, although the effects of amygdalofugal input on neural coding of taste information is not well understood. The present study examined responses of 110 NST neurons to 15 taste stimuli before, during, and after electrical stimulation of the CeA in rats. The taste stimuli consisted of different concentrations of NaCl (0.03, 0.1, 0.3 M), sucrose (0.1, 0.3, 1.0 M), citric acid (0.005, 0.01 M), quinine HCl (0.003, 0.03 M), and 0.03 M MSG, 0.1 M KCl, as well as 0.1 M NaCl, 0.01 M citric acid, and 0.03 M MSG mixed with 10 μM amiloride. In 66% of NST cells sampled (73/110) response rates to the majority of effective taste stimuli were either inhibited or augmented. Nevertheless, the magnitude of effect across stimuli was often differential, which provides a neurophysiological mechanism to alter neural coding. Subsequent analysis of across-unit patterns showed that amygdalofugal input plays a role in shaping spatial patterns of activation and could potentially influence the perceptual similarity and/or discrimination of gustatory stimuli by altering this feature of neural coding.

INTRODUCTION

In rodents the rostral zone of the nucleus of the solitary tract (NST) is the first central synapse for gustatory afferent fibers that innervate taste buds of the oral cavity. Neurons in the NST have two major targets: 1) oral premotor neurons in the underlying medullary reticular formation and 2) the pontine parabrachial nucleus (PBN) that in turn distributes taste information indirectly via the thalamus to the insular cortex (IC) and directly to the lateral hypothalamus (LH), central nucleus of the amygdala (CeA), and bed nucleus of the stria terminalis (BNST) (Halsell et al. 1996; Norgren 1976; Norgren and Leonard 1971; Travers and Norgren 1983). In addition to these ascending pathways and local medullary connections, the NST and PBN receive innervation from IC, CeA, BNST, and LH that support top-down regulation of neural processing (Halsell 1998; Moga et al. 1990a; Veening et al. 1984; Whitehead et al. 2000). In fact, hindbrain neural processing in isolation from the forebrain (i.e., decerebrate preparation) is not sufficient to support learned and some forms of unlearned control of taste preference/aversion behavior (Grill and Norgren 1978; Grill et al. 1986). One view is that centrifugal inputs alter neural encoding of sapid stimuli and thus play a significant role in the establishment and elaboration of taste preference that can promote or discourage consumption.

In addition to taste quality and intensity, the responses of brain stem taste neurons are differentially affected by prior experience and physiological state (Chang and Scott 1984; Foster et al. 1996; Hajnal et al. 1999; Jacobs et al. 1988; McCaughey and Scott 2000; McCaughey et al. 1996, 1997; Nakamura and Norgren 1995; Shimura et al. 1997a,b). For instance, a common finding after acquisition of a conditioned taste aversion is avoidance of the conditioned taste stimulus coincident with an enhanced neural response to that stimulus. Following induction of a negative body sodium balance, the common finding is exaggerated intake of sodium salt, particularly at higher concentrations that are normally avoided, coincident with decreased sensitivity of hindbrain taste cells to sodium salt. Intraduodenal lipid infusion, which reduces intake of sucrose, selectively suppresses neural responses to sucrose in the PBN. Importantly, inhibition and excitation of hindbrain taste responsive neurons can also be produced by stimulation of IC, BNST, CeA, and LH.

In the NST, the most common effect of CeA (91–93%) and LH (66–100%) stimulation was excitatory; BNST stimulation was predominantly inhibitory (80%), whereas IC activation induced a more equal distribution of excitation (47%) and inhibition (53%) (Cho et al. 2002, 2003; Di Lorenzo and Monroe 1995; Li et al. 2002; Matsuo et al. 1984; Murzi et al. 1986; Smith and Li 2000; Smith et al. 2005). One synapse further along in the ascending gustatory system, forebrain activity produced a somewhat different pattern of effects on taste cells in the PBN. Here activation of the BNST was entirely inhibitory; CeA (85%) and IC (71%) most often produced inhibition, whereas inhibition and excitation occurred equally often during LH stimulation (Di Lorenzo and Monroe 1992; Li and Cho 2006; Li et al. 2005; Lundy Jr and Norgren 2001, 2004). Only a few of these studies have thoroughly characterized the influence of descending forebrain inputs on taste-evoked responses and thus provide insight into the effects on neural encoding.

One theory of gustatory neural coding emphasizes that individual cells respond to several different compounds and stimulus identity and valence are encoded in the pattern of activation of large ensembles of neurons with distinct response profiles (for a critical review, see Specter and Travers 2005). In fact, prior research has shown that descending input from IC can influence the population code for normally preferred stimuli, such as sucrose and saccharin in the NST and PBN (Di Lorenzo 1990; Di Lorenzo and Monroe 1995). Following
Amygdala stimulating electrodes

The rats were anesthetized with a 50 mg/kg intraperitoneal (ip) injection of pentobarbital sodium (Nembutal). Additional doses of Nembutal (10 mg/kg) were administered as necessary to continue a deep level of anesthesia. Rectal temperature was monitored throughout recording sessions. The animals were secured in a stereotaxic instrument using non-puncture ear bars and a bite bar. Two stainless steel electrodes, insulated except at the tips, were lowered into CeA using the following coordinates relative to bregma and the interparietal bone: P = 2.0 mm, L 4.0 mm, and V 8.5 mm. A hole was drilled through the bone at the P-L coordinates and the dura excised. The stimulating electrodes were lowered through the cortical tissue using a custom made jig. Electrical impulses delivered to the CeA were timed with a Grass S48 train generator attached to an isolated constant-current source. Stimulation parameters were 1-s trains of 10 pulses delivered at 0.5 Hz for 10 s (total of 50 pulses). The duration and amplitude of an individual pulse was 0.2 ms and 0.4 mA, respectively.

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Localizaon of NST gustatory neurons

Seven days after surgery, each rat was reanesthetized (Nembutal, 35 mg/kg ip) and mounted in the stereotaxic device using the fake ear bars and the NST was located electrophysiologically. After drilling a small hole through the interparietal bone, a glass-coated tungsten microelectrode (1.5–3.0 MΩ at 1 kHz) was lowered through the hole using a microdrive. For access to the NST, the electrode was lowered to coordinates ranging from 12.0 to 12.6 mm posterior to bregma and 1.4 to 2.0 mm lateral to the midline according to the rat stereotaxic atlas (Paxinos and Watson 1982). Typically, taste-evoked neural activity was encountered 6.4–7.0 mm ventral to the surface of the cerebellum. Any neuron was tested for its sensitivity to 0.1 M NaCl applied to the anterior two thirds of the tongue. If a response was not obtained with NaCl, then 0.3 M sucrose, 0.01 M citric acid (CA), and 0.003 M quinine hydrochloride (QHCl) were tested. Neural activity was amplified (×1,000) and fed through an artifact suppressor. The artifact suppressor was used to dampen the electrical stimulus artifact in the neural signal. Cambridge Electronic Design’s Spike2 hardware and software were used to convert the neural data to digital format and to analyze neural responsiveness. Most NST recording sessions occurred on the side of CeA electrode implantation due to the primarily ipsilateral nature of amygdalofugal input (Halsell 1998; van der Kooy et al. 1984; Whitehead et al. 2000). Nevertheless, some sessions (20 of 84) recorded NST taste responses on the side contralateral to CeA stimulation. After each recording session, the exposed brain was covered with nontoxic silicone elastomer (WPI) and then covered with two-component epoxy (ITW).

Stimulus delivery and protocols

A computer-controlled delivery system was used for taste stimuli and water presentation to the anterior tongue. Fifteen taste stimuli were presented during recordings from each neuron, which consisted of different concentrations of NaCl (0.03, 0.1, 0.3 M), sucrose (0.1, 0.3, 1.0 M), CA (0.005, 0.01 M), and QHCl (0.003, 0.03 M) as well as 0.03 M MSG, 0.1 M KCl, and 0.1 M NaCl, 0.01 M CA, and 0.03 M MSG mixed with 10 μM amiloride. The rationale for adding amiloride to NaCl, MSG, and citric acid was: 1) amiloride blocks the response of a specific class of gustatory cells to NaCl and MSG, but not citric acid (Formaker et al. 2004; Lundy Jr and Contreras 1999), and 2) amiloride alters the perceptual qualities of NaCl and MSG in rodents (Spector et al. 1996; Stapleton et al. 2002). Stimulus order varied from one recording session to another. Each of these 15 taste stimuli was applied to the tongue using a water–stimulus–water sequence with a 10-s application of water followed by tastant application for 10 s. The water flow that followed tastant application was for 30 s and the total time between different stimulus applications was 60 s. In most recording sessions, this array of taste stimuli was applied three times: two control and one test application. The stimulation protocol was as follows: control series 1–test series–control series 2. Tastant application during a control series was without CeA stimulation, whereas the test series consisted of fluid application during concurrent electrical stimulation of the CeA. Prior to the test series, baseline activity in the absence of fluid flowing over the tongue was recorded during CeA stimulation.
Data analysis

Corrected neural responses were calculated by subtracting the preceding 10-s discharge rate to water from the taste response. When a neuron was tested with control series 1 and 2, these response rates were averaged. A response to sapid stimulation was considered significant when the Z value was \(< -2\) or \(> +2\) (uncorrected taste discharge – preceding water discharge/SD of preceding water discharge).

During the test series, the five trains of electrical pulses were delivered in seconds 1, 3, 5, 7, and 9 of the concurrent 10-s sapid stimulus application (ON SECONDS). No brain stimulation occurred in seconds 2, 4, 6, 8, and 10 (OFF SECONDS). For each of the control and test series, the mean corrected discharge rates for “ON SECONDS” and “OFF SECONDS” were calculated. During each of these response periods, a difference score was calculated by subtracting the test series from the control series (absolute change in neural discharge). The resultant difference score was also divided by the SD of the control series rates to obtain the Z value. Neurons in the present study were classified as inhibited or augmented if the resultant Z value for \(\geq 30\%\) of the sapid stimuli was \(< -2\) or \(> +2\), respectively. The breadth of responsiveness was calculated according to the formula

\[
H = -1.661 \left( \sum_{i=1}^{4} P_i \log P_i \right)
\]

where \(P_i\) is the proportion of the response to each of the stimuli against the total response to all the stimuli (Smith and Travers 1979).

The noise-to-signal \((N/S)\) ratio of each neuron was calculated according to the formula \(N/S = R_2/R_1\), where \(R_2\) is the response rate to the second-best stimulus and \(R_1\) to the most effective stimulus (Spector and Travers 2005).

Data were analyzed by factorial and repeated-measures ANOVAs and one-sample \(t\)-test. In some instances, post hoc contrast analyses (Bonferroni) were used to determine the source of statistically significant differences. The results are shown as the means ± SE. The Pearson’s product-moment correlation coefficients for all possible pairs of responses were calculated and used to conduct cluster analyses (average between-group linkage method). This analysis was used to categorize individual neurons with similar response characteristics. The Neural Discrimination Index \((\delta, \text{delta})\) for all possible pairs of responses was calculated according to the formula

\[
\delta = \left[ \frac{\sum_{i=1}^{n} (v_i - w_i)^2}{\sum_{i=1}^{n} v_i^2 + \sum_{i=1}^{n} w_i^2} \right]^{1/2}
\]

where \(v_i\) and \(w_i\) are the responses of the \(i\)th neuron to stimulus \(V\) and \(W\), respectively. The ratio reflects the overall similarity of the across-neuron pattern produced by pairs of taste stimuli in terms of absolute effectiveness and distribution of action potentials across the population of cells sampled (Di Lorenzo 1989, 1990; Di Lorenzo and Monroe 1995). To compare stimulus relationships before and during CeA activation, the matrix of delta values for each condition was used to conduct multidimensional scaling (Euclidean distances method).

All data analyses were done using PASW (Predictive Analytics SoftWare) statistics 17.0 and statistical differences were reported only if alpha \(< 0.05\) was met.

Histological processing and analyses

At the end of an experiment, the rats were given a lethal dose of Nembutal (150 mg/kg ip) and perfused intracardially with 0.9% saline followed by 4% paraformaldehyde. The brain was removed, cut coronally in 50 \(\mu\)m sections using a freezing microtome, and stained with cresyl violet. The stimulating electrode tracks were localized using a light-field microscope.

RESULTS

Histology

The neurophysiological data in the present study were obtained from animals in which the stimulating electrodes were histologically confirmed to terminate in the CeA. The representative photomicrographs in Fig. 1, A, B, C, and D show a stimulating electrode track at different stereotaxic coordinates in the CeA.

Neuronal categorization

In all, 110 single NST taste neurons were tested before, during, and after electrical stimulation of the CeA and while responding to 15 sapid stimuli; 91 ipsilateral and 19 contralateral to the CeA stimulating electrodes. Pearson’s product-
moment correlation coefficients for all possible pairs of responses to the 15 taste stimuli were generated and used to conduct cluster analysis. The level at which two neurons or groups of neurons connect in a dendrogram indicates their overall similarity in responsiveness. Numbers near 0 refer to neurons or groups of neurons with a high degree of similarity, whereas larger numbers refer to those with a lower degree of similarity. The dendrogram of Fig. 2 shows that four major groups dominated this population of NST taste cells: 56 neurons classified as NaCl-best (N), 24 as sucrose-best (S), 25 as citric acid-best (C), and 5 as QHCl-best (Q). The mean corrected response rates of each best-stimulus cell group plotted as a function of the 15 stimuli are shown in Fig. 3. A (sucrose), B (NaCl), C (MSG), D (citric acid), and E (QHCl and KCl).

Briefly, separate one- or two-factorial ANOVAs revealed that each cell type responded in a concentration-dependent manner to sucrose, NaCl, and QHCl, but not to citric acid. Comparison of response rates between cell types revealed that S-best cells carried the greatest neural activity elicited by sucrose, with the order of stimulus effectiveness being S-best > N-best = C-best > Q-best. For NaCl solutions alone, N-best and C-best neurons were most responsive compared with S-best and Q-best. Addition of amiloride to 0.1 M NaCl significantly suppressed neural activity in S- and N-best cells, but not in C- and Q-best cells, which altered the order of sensitivity to C-best > Q-best = N-best > S-best. The bulk of neural activity evoked by citric acid, QHCl, and KCl was carried by C-best cells. Finally, the order of effectiveness for the single concentration of MSG was N-best = C-best > S-best > Q-best. Addition of amiloride to MSG significantly suppressed neural activity in N-best cells, but not in S-, C- and Q-best cells, which altered the order of sensitivity to C-best > S-best > N-best > Q-best.

Amygdala stimulation

Of the 110 NST neurons recorded, electrical stimulation of the CeA facilitated taste elicited firing rates in 45 neurons, inhibited firing rates in 28, and was without effect in 37 (Table 1). Of the neurons influenced by CeA, ipsilateral recordings resulted in a greater percentage of neurons under excitatory control compared with contralateral recordings, while NST cells under inhibitory control were more equal distributed. Overall, nearly threefold more N-best neurons were augmented by CeA stimulation than inhibited, whereas inhibition and excitation was more equally distributed in C-best and S-best cells. Only one of the five Q-best neurons was influenced by CeA activation where response rates were inhibited. Figure 4 shows an example of CeA-induced augmentation of taste-evoked responses in an S-best neuron. Visual inspection indicates that responses to most stimuli were increased by CeA stimulation. Across all 45 augmented cells, the mean percentage of stimuli showing increased effectiveness was 61.8 ± 3.1%, whereas that showing decreased effectiveness was 1.7 ± 0.7%. For the cells under inhibitory control, 50.3 ± 2.9% of the stimuli showed reduced effectiveness and 3.3 ± 1.1% increased effectiveness. Thus with few exceptions, the majority of taste-elicited responses of individual cells were either excited or inhibited.

NaCl-best neurons

EXCITATION. For 20 of 27 N-best cells augmented by CeA activation, taste-evoked responses to each taste stimulus were recorded before (open circles) and after (filled circles) electrical stimulation of the CeA (Fig. 5A). The discharge rates were nearly identical, indicating that the recording was stable over
FIG. 3. Mean control responses (±SE) of NST neurons to 15 taste stimuli as a function of best-stimulus category. Open bars correspond to S-best cells; lightly shaded, N-best; moderately shaded, C-best; and solid black, Q-best. For each stimulus concentration, statistical analyses are represented by letters where only those bars with the same letter are not statistically different at alpha < 0.05. Where appropriate, bold italic letters represent a statistically larger response compared with the lowest concentration of a particular stimulus, whereas underlined letters correspond to a statistically smaller response when amiloride was added to a given stimulus.
time and CeA stimulation had no long-lasting effect on gustatory responsiveness. Shown in Fig. 5B are the mean responses to the same taste stimuli during the “ON SECONDS” (e.g., 1, 3, 5, 7, and 9) of the control (open circles) and CeA test series (filled circles). A significant interaction between application condition and sapid stimulus was evident. Post hoc analyses using Bonferroni adjustment for multiple comparisons revealed that CeA activation significantly increased the response rate evoked by each taste stimulus, which persisted, albeit to a lesser degree, during the “OFF SECONDS” when no brain stimulation occurred (e.g., 2, 4, 6, 8, and 10; Fig. 5C).

The difference in response rate between control and CeA test applications is shown in Fig. 5D. A separate one-tailed t-test (test value = 0) confirmed a significant enhancement in response rate and spontaneous activity during CeA activation. Comparisons of the magnitude of excitation induced by CeA activation revealed a significant main effect for stimulus. Augmentation of taste-evoked responses was greater for NaCl, the most effective stimulus for N-best cells, and MSG compared with sucrose, citric acid, QHCl, and KCl, as well as NaCl and MSG mixed with amiloride.

<table>
<thead>
<tr>
<th>Best Stimulus</th>
<th>Inhibited</th>
<th>Enhanced</th>
<th>Unaffected</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (n = 56)</td>
<td>6 (11%)</td>
<td>26 (46%)</td>
<td>20 (36%)</td>
</tr>
<tr>
<td>Citric acid (n = 25)</td>
<td>6 (24%)</td>
<td>6 (24%)</td>
<td>4 (16%)</td>
</tr>
<tr>
<td>Sucrose (n = 24)</td>
<td>4 (17%)</td>
<td>9 (38%)</td>
<td>6 (25%)</td>
</tr>
<tr>
<td>QHCl (n = 5)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Total (n = 110)</td>
<td>16 (14%)</td>
<td>41 (37%)</td>
<td>34 (31%)</td>
</tr>
</tbody>
</table>

The number of neurons that were inhibited, excited, and unaffected by electrical stimulation of the CeA are listed as a function of neuron type and laterality of NST recording site.
Low-frequency stimulation of CeA (10 pulses/s) elevated spontaneous activity by 3.1 spikes/s, which remained elevated by 2.4 spikes/s during the “OFF SECONDS” when no brain stimulation occurred. Together with the lack of evidence for direct projections from gustatory NST to CeA would suggest that the effects observed on taste-elicited responses were not due to antidromic activation.

INHIBITION. For eight of nine N-best cells inhibited by CeA activation, evoked responses to each taste stimulus were recorded before and after electrical stimulation of the CeA and were stable over time $\left[F_{(1,7)} = 0.13, P = 0.72; \text{not shown}\right]$. During the “ON SECONDS” (Fig. 6A), a significant interaction between application series and sapid stimulus was observed. Post hoc analyses revealed that CeA activation decreased the responses to MSG and one or more concentrations of sucrose, NaCl, citric acid, and QHCl. Unlike CeA-induced excitation, however, the inhibitory influence did not persist during the “OFF SECONDS” when no brain stimulation occurred $\left[F_{(1,8)} = 0.51, P = 0.49; \text{not shown}\right]$. This suggests that the inhibitory effect of CeA activation was rapidly reversed. Using our definition of a significant taste response (i.e., $Z < -2$ or $> +2$) from the mean response to water, stimulation of the amygdala reduced the effectiveness of nonbest stimuli by 29% (Table 2). The forebrain activation had a much smaller effect on responses to NaCl in cells that did not respond best to this stimulus (7%).

The difference in response rate between control and CeA test applications is shown in Fig. 6B. A separate one-tailed t-test (test value = 0) confirmed a significant reduction in response rate to most stimuli during CeA activation. A significant main effect for stimulus also was observed with the magnitude of inhibition being greatest for 0.3 M NaCl compared with all other stimuli, except MSG and the two lower concentrations of NaCl. The spontaneous activity was not significantly affected by CeA stimulation. This group of N-best cells also was significantly less responsive to NaCl and MSG compared with the N-best neurons augmented by CeA activation (compare Figs. 5B and 6A).

C-best neurons

EXCITATION. Evoked responses to each taste stimulus were recorded in six of eight augmented C-best cells before and after electrical stimulation of the CeA and were stable over time $\left[F_{(1,5)} = 0.03, P = 0.85; \text{not shown}\right]$. Post hoc analyses of the significant interaction between application series and sapid
stimulus showed that evoked responses to most of the taste stimuli were higher during concurrent CeA activation (Fig. 7A). The excitatory influence persisted during the “OFF SECONDS” when no brain stimulation occurred \((F(14,98) = 3.84, P < 0.01; \text{not shown})\). The difference between control and test responses during the “ON SECONDS” is shown in Fig. 7C. Responses to NaCl were increased to a greater degree compared with sucrose, MSG, QHCl, and KCl. The spontaneous activity was not significantly elevated by CeA stimulation.

The lack of evidence for direct projections from gustatory NST to CeA and inability to follow low-frequency stimulation suggest that the excitatory effects observed on taste-elicited responses were not due to antidromic activation (Li et al. 2002).

### INHIBITION

Again, the control discharge rates to sapid stimulation in C-best neurons (eight of ten) inhibited by CeA activation were stable over time \([F(1,7) = 1.13, P = 0.32; \text{not shown})\]. The evoked response to each of the taste stimuli was lower during concurrent CeA activation compared with control responses (Fig. 7B). The inhibitory influence, however, did not persist during the “OFF SECONDS” when no brain stimulation

**TABLE 2. Reduction of taste-evoked responses by CeA stimulation**

<table>
<thead>
<tr>
<th>Neuron Type</th>
<th>NaCl</th>
<th>Citric Acid</th>
<th>Sucrose</th>
<th>Quinine</th>
<th>MSG</th>
<th>KCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Best</td>
<td>0.03</td>
<td>0.005</td>
<td>0.003</td>
<td>0.03</td>
<td>0.03</td>
<td>0.1</td>
</tr>
<tr>
<td>A-Best</td>
<td>0.1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>S-Best</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
<td>0.1</td>
</tr>
</tbody>
</table>

This table shows the number of neurons that responded significantly to NaCl, citric acid, sucrose, QHCl, MSG, and KCl during the “On Seconds” of the control (denominator) and test applications (numerator), listed as a function of neuron type. The denominators in bold reflect the best stimulus for each of the three cell types and the total number of neurons deemed inhibited by stimulation of the CeA. The percentage reduction of responses to each sapid stimulus across neuron types is shown on the bottom and that of the non-best responses within a neuron type on the right.
occurred \( F(1,9) = 2.99, P = 0.11; \) not shown. A separate one-tailed \( t \)-test (test value \( = 0 \)) confirmed a significant reduction in response rate to most stimuli during CeA activation (Fig. 7D), but not spontaneous activity. Unlike N-best cells, the magnitude of CeA-induced inhibition in C-best neurons did not differ significantly across sapid stimuli. Nevertheless, forebrain stimulation reduced the effectiveness of nonbest stimuli by 17% (Table 2). When considering only the lowest concentrations of sucrose and QHCl, responsiveness of C-best cells was reduced 43%.

**S-best neurons**

**EXCITATION.** For eight of ten S-best cells augmented by CeA activation, evoked responses to each taste stimulus were recorded before and after electrical stimulation of the CeA and were stable over time \( F_{(1,7)} = 0.36, P = 0.56; \) not shown. Figure 8A shows that activation of the CeA increased the responses to taste stimuli during the “ON SECONDS.” Although not shown, enhanced responsiveness persisted during the “OFF SECONDS” \( F_{(1,12)} = 3.9, P < 0.01 \) to most stimuli except QHCl and KCl \( (P \) values \( \geq 0.29 \)). The difference between control and test responses during the “ON SECONDS” is shown in Fig. 8C. A separate one-tailed \( t \)-test (test value \( = 0 \)) confirmed a significant enhancement in response rate to all stimuli during CeA activation. In general, the magnitude of excitation tended to be larger for NaCl and high concentrations of sucrose, although only 0.3 M NaCl differed significantly from the other qualitatively distinct stimuli.

**INHIBITION.** The control taste responses were also stable in the eight sucrose-best neurons inhibited by CeA activation \( F_{(1,7)} = 2.1, P = 0.18; \) not shown. The evoked response to sucrose and NaCl was lower during concurrent CeA activation compared with control responses (Fig. 8B). Although a significant interaction between factors was observed for the “OFF SECONDS” when no brain stimulation occurred \( F_{(14,98)} = 2.7, P < 0.01; \) not shown, post hoc analyses revealed that inhibition persisted only for responses to 0.3 M sucrose and 0.1 M NaCl/amiloride. Figure 8D shows the difference between control and test responses during the “ON SECONDS.” The magnitude of inhibition was greater...
for sucrose compared with that for MSG, citric acid, QHCl, and KCl, but not NaCl. Inhibition of NaCl responses was significantly greater compared with that of QHCl and KCl. Spontaneous activity was not significantly inhibited by CeA activation. The effectiveness of nonbest stimuli to activate S-best cells was reduced by 20% during CeA activation (Table 2). The forebrain stimulation had a much greater effect when considering only QHCl responsiveness, reducing it by 57%.

Similar to N-best cells, significant differences in taste stimulus responsiveness was observed between S-best neurons augmented by CeA activation compared with those inhibited. Specifically, the control taste-evoked responses in the augmented S-best cells were lower in magnitude than responses to the same stimuli in the inhibited S-best cells (compare open circles in Fig. 8, A and B).

Breadth of tuning

To facilitate comparison with previous studies, the breadth of tuning was calculated using responses to 0.3 M sucrose, 0.1 M NaCl, 0.01 M citric acid, and 0.003 M QHCl. Two measures were calculated—entropy and noise-to-signal ratio (N/S)—each representing response specificity of a neuron. The entropy value ranges from 0 to 1. A value of 0 corresponds to a neuron that was activated by only one stimulus; a value of 1, to a neuron activated equally by all stimuli. The N/S ratio also ranges from 0 to 1. Lower N/S indicates a greater difference in response magnitude between the best and next-best stimulus.

Table 3 lists the entropy and N/S values during the “ON SECONDS” of the control and test applications as a function of neuron type and response category (e.g., inhibited or facilitated). All cell types contained neurons that were inhibited or augmented during CeA activation, although significant changes were not observed for entropy values or N/S ratios compared with control conditions. Thus both measures indicate that amygdalofugal influence did not alter stimulus selectivity of NST taste cells. This is despite moderate reductions in the ability of side-band stimuli to significantly increase neural activation above baseline level (Table 3).

Cluster analysis

Another way to compare the relationships among neurons in the two samples is hierarchical cluster analysis. Pearson’s product-moment correlation coefficients were generated for all possible pairs of responses to the 15 taste stimuli in the 28 neurons inhibited and the 45 augmented by CeA activation. In general, the dendrograms produced by the control and test applications were quite similar for the population of cells inhibited (Fig. 9, A and B) or augmented (Fig. 9, C and D) by

FIG. 8. Data from the S-best neurons that were augmented or inhibited by CeA stimulation. A: the response rates of augmented cells during stimulation of the CeA (filled circles) and during the corresponding control time periods in trials without CeA stimulation (open circles). B: the response rates of inhibited cells during stimulation of the CeA (filled circles) and during the corresponding control time periods in trials without CeA stimulation (open circles). C: the difference between the response rates shown in A. D: the difference between the response rates shown in B. The effect of the pulse trains on spontaneous activity during the same time periods is also shown in C and D (Sp). Stimuli: sucrose: 0.1 M (S1); 0.3 M (S2); 1 M (S3); NaCl: 0.03 M (N1); 0.1 M (N2); 0.3 M (N3); 0.1 M mixed with amiloride (N2/amil); MSG: 0.03 M (MSG); 0.03 M mixed with amiloride (MSG/amil); citric acid: 0.005 M (CA1); 0.01 M (CA2); 0.01 M mixed with amiloride (CA2/amil); QHCl: 0.003 M (Q1); 0.03 M (Q2); KCl: 0.1 M (K). *Significantly different from control series or significantly different from 0.
KCl and QHCl remained closely associated with citric acid. Closer between sucrose and NaCl (Fig. 11, MSG and NaCl. Again, at longer response times MSG moved NaCl, whereas KCl and QHCl were more separated from citric increased, however, MSG moved closer between sucrose and all other normally nonpreferred stimuli. As response duration NaCl and KCl. MSG remained somewhere between NaCl and the other tastants in the space match, as closely as possible, the given separation between sucrose and all other taste stimuli was evident within the first second (Fig. 10, CeA activation. Each included three clusters that were dominated by a single best-stimulus category. Nevertheless, the inhibitory effect of CeA activation seemed to decrease the average similarity in tantant responsiveness between pairs or groups of neurons. That is, the distance within and between clusters increased. In contrast, the excitatory effect of CeA activation appeared to increase similarity in tantant responsiveness, particularly for S-best cells. Moreover, four neurons switched groups during concurrent CeA stimulation. Two neurons in the S cluster (38 and 42) and one in the C cluster (24) during control applications were grouped with N cluster cells during CeA test applications, whereas a single N cluster cell (10) switched grouping to the C cluster.

**Multidimensional scaling**

To investigate taste stimulus relationships, delta values for all possible pairs of stimuli across the neuronal samples were calculated for control and CeA test applications during second 1 of the taste response, seconds 1 and 3, and seconds 1, 3, 5, 7, and 9. A delta value of 0 corresponds to a pair of stimuli that evoked an identical pattern of activity across neurons; a value of 1, to stimuli that activated completely different subsets of cells or when one of the stimuli in the pair never evoked a response. The delta value matrices were used for multidimensional scaling (MDS) that assigned the stimuli to specific locations in a conceptual space such that the distances between tastsants in the space match, as closely as possible, the given dissimilarities in the pattern of neural activation they produced. Based on taste responses inhibited by CeA activation, greater separation between sucrose and all other taste stimuli was evident within the first second (Fig. 10, A and B). Citric acid and QHCl remained close together, but further away from NaCl and KCl. MSG remained somewhere between NaCl and the other normally nonpreferred stimuli. As response duration increased, however, MSG moved closer between sucrose and NaCl, whereas KCl and QHCl were more separated from citric acid (Fig. 10, C and F).

Based on taste responses augmented by CeA activation, sucrose stimuli moved closer together, but further away from all other stimuli during the first second (Fig. 11, A and B). Citric acid, QHCl, and KCl moved closer to one another as did MSG and NaCl. Again, at longer response times MSG moved closer between sucrose and NaCl (Fig. 11, C and F). However, KCl and QHCl remained closely associated with citric acid. These data indicate that amygdalofugal influence can alter the spatial pattern of neural activation as early as the first second of the response.

**DISCUSSION**

The present experiment replicates a previous one examining amygdalofugal influence on gustatory neurons in the NST (Li et al. 2002). Although conducted in a different species, hamster, Li and colleagues (2002) showed that CeA stimulation predominantly excited taste-responsive neurons and, in a subset of cells tested, augmented taste-evoked responses. Similar to the present study there did not appear to be any clear chemical specificity in enhancement of taste responses. Despite this uniform effect, other aspects of the results differed between these studies. First, we observed that twice as many NST gustatory neurons were under amygdalofugal control in rat (73/110, 66%) than those in hamster (36/109, 33%). Although excitation predominated overall, a large number of taste cells were inhibited by CeA stimulation (25% of total neurons sampled) and the distribution of excitatory and inhibitory effects varied across neuron types. Specifically, threefold more N-best neurons were under excitatory control compared with inhibitory, whereas excitation and inhibition occurred equally often in S-best and C-best cells. Amygdalofugal-induced inhibition of taste neurons in the NST of hamster was rarely observed (3% of total neurons sampled). In addition to species differences, other experimental variables must be considered in accounting for these differences, such as stimulating electrodes (a single concentric bipolar electrode vs. two individual stainless steel wires) and electrical stimulation parameters (0.5 ms, ≤0.1 mA, 1/3 Hz vs. 0.2 ms, 0.4 mA, 10 Hz). Nevertheless, it is clear that taste neurons in the brain stem are subject to top-down regulation. The present experiment further elaborates how CeA influences the neural code for taste stimuli in the NST by using a wide range of qualitatively distinct taste stimuli and increasing the number of neurons tested during tantant application and concurrent CeA activation.

**NST gustatory processing: response profiles**

In several ways the present experiment was consistent with previous ones examining the response characteristics of NST taste cells to a similar array of sapid stimuli in awake behaving rats (Nakamura and Norgren 1991, 1993, 1995). Each found
Inhibited NST Cells

Control

CeA Test

Augmented NST Cells

Control

CeA Test

FIG. 9. Dendrograms depicting the relative response similarity among NST neurons inhibited or augmented by CeA activation during control and test applications. In the body of each dendrogram, the notations N, S, and C correspond respectively to NaCl-best, sucrose-best, and citric acid-best neurons. The number of each neuron is listed on the left to aid comparison. *Denotes neurons that changed cluster grouping during concurrent CeA activation.
that neurons were segregated into four clusters dominated by best-stimulus class; N-best (NaCl), S-best (sucrose), C-best (citric acid), and Q-best (QHCl). Both N-best and S-best cell types responded in a concentration-dependent manner to NaCl and sucrose and were sensitive to MSG. These cells were driven only moderately, if at all, by citric acid, QHCl, and KCl. Despite these similarities, striking differences were observed in the stimulus sensitivity of C-best and Q-best neurons. In awake-behaving rats, these cell types were more narrowly tuned, being rather insensitive to NaCl and KCl, whereas the present sample of C-best and Q-best cells was quite sensitive to these two sapid stimuli. We also observed that MSG was as potent a stimulus for C-best neuron types as it was for N- and S-best cells. It should be noted that comparison between Q-best response profiles is somewhat lessened by the small sample size in previous studies and the present one.

If one assumes that pentobarbital anesthesia reduces overall activity of the forebrain, then one might expect that electrical stimulation would artificially restore activity that has been pharmacologically suppressed and, consequently, sharpen the responses of NST taste neurons to sapid stimuli similar to that observed in awake-behaving animals. However, amygdalofugal influence had minimal impact on the specificity of NST taste cells for a particular category of chemical(s), as measured by the breadth of responsiveness (entropy) and N/S ratio. This contrasts with the amygdalofugal influence on PBN taste-evoked activity, using the same four basic stimuli, electrical stimulation parameters, and species (Lundy Jr and Norgren 2001, 2004).

In both of the earlier studies, CeA stimulation significantly reduced the entropy value of each PBN taste cell type, indicating that they became more selective in their sensitivity to sapid stimuli. Both a smaller degree of inhibition of NST neural responses to side-band stimuli and less stimulus specificity of enhanced responsiveness compared with PBN might account for this difference. That is, the degree of PBN taste cell inhibition was large enough to decrease the number of N-best, C-best, and S-best neurons that responded significantly above spontaneous activity to nonbest stimuli by 65, 45, and 38%, respectively. The corresponding values in NST were 29, 16, and 20% for N-best, C-best, and S-best neurons, respectively. Moreover, CeA-induced excitation of taste activity in PBN was limited to the response of N-best neurons to NaCl; the re-
sponses to other classes of sapid stimuli were unaffected. In NST, amygdalofugal facilitation of neural activity was more general in nature, affecting different cell types and qualitatively distinct taste stimuli.

Amygdalofugal influence on NST taste cells did appear to alter the average similarity in tastant responsiveness between pairs of neurons. Visual inspection of the dendrogram for NST neurons under inhibitory control suggests that neurons within a particular best-stimulus category became more dissimilar in their pattern of tastant responsiveness during CeA stimulation. This might be the result of variable suppression of responses to different side-band stimuli across cells. In contrast, similar analyses for NST cells under excitatory control indicated that CeA activation increased the similarity in sapid stimulus responsiveness, particularly for S-best cells. Thus amygdalofugal facilitation produced greater homogeny in S-best neuron responsiveness to a range of qualitatively distinct tastants.

Stimulus relationships

Behavioral generalization studies demonstrate that sucrose, NaCl, citric acid, and quinine are representative of independent perceptual taste qualities (Grobe and Spector 2008; Yamamoto et al. 1991). These distinct perceptual qualities are well represented by the response properties of NST taste cells even in the absence of CeA activation. Nevertheless, descending inhibitory and excitatory modulation did alter the spatial representation of some taste stimuli. For instance, MSG has been shown to be perceptually similar to NaCl and sucrose, but distinct from citric acid and QHCl (Grobe and Spector 2008). During CeA-induced inhibition, MSG in multidimensional taste space moved closer to NaCl and sucrose, indicating a more similar across-neuron pattern. Amygdalofugal excitation also increased the similarity in the distribution of NaCl and MSG elicited action potentials across units. In contrast, the spatial code associated with KCl was altered in a manner more consistent with behavioral dissimilarity rather than similarity. Perceptually, KCl has quinine-like and citric acid-like qualities, although rats can discriminate between these stimuli (Grobe and Spector 2008; St John and Spector 1998). The movement of KCl away from citric acid and high-concentration QHCl in taste space, particularly for cells under inhibitory control, suggests that CeA activation increased the distinctive-
noss of the across-neuron pattern produced by KCl and, by inference, discriminability. Using the same logic, one would also predict that KCl is behaviorally distinct from amiloride adulterated NaCl because the pattern of neural activation produced by the NaCl/amiloride solution was nearly identical to that of citric acid. However, in short-term intake tests, rats are unable to discriminate between KCl and NaCl mixed with amiloride (Spector et al. 1996).

A lack of full correspondence between behavior and the activity of NST taste cells during CeA activation is not surprising, given that neural control of behavioral outcome may arise from modulation at different levels of the central taste system. Temporal patterns of activation not examined in the present study also likely contribute to the gustatory neural code. Nevertheless, the present results do suggest that activation of amygdalofugal inputs could influence the perceptual similarity and/or discrimination of gustatory stimuli by altering one feature of neural coding: spatial patterns of activation. One might further speculate that under awake-behaving conditions there would be more discrete context-dependent activation of CeA neurons that facilitate and/or inhibit taste cells and thus more specific changes in chemical sensitivity. Forebrain-mediated facilitation and inhibition of taste responses might also influence temporal patterns of activation and remain undetected using the present experimental procedures.

The functional association between brain stem and forebrain taste areas has been clear for decades, although the nature of the association in terms of neural coding remains cloudy. This is because the neurochemical mediators of descending forebrain modulation remain unknown, as do their effects on taste-guided behavior. We do know that multiple neurotransmitters can influence activation of NST taste cells, including glutamate (Li and Smith 1997; Wang and Bradley 1995), substance P (Davis and Smith 1997; King et al. 1993), γ-aminobutyric acid (GABA) (Smith et al. 1998), and enkephalin (Li et al. 2003; Moga et al. 1990b) to name a few, although very little is known concerning their involvement in centrifugal regulation. A single study provides evidence that IC-induced inhibition of NST taste-responsive cells involves GABAergic signaling (Smith and Li 2000). Whether this is a monosynaptic or polysynaptic influence remains unknown, although indirect evidence based on response latencies following IC activation is consistent with the former. Yet other studies examining the influence of stimulating different forebrain regions, such as amygdala and hypothalamus, on NST taste cells report response latencies suggestive of both monosynaptic and polysynaptic connections (Cho et al. 2002; Li et al. 2002).

Even less is known about the neurotransmitters that control activation of taste neurons in the PBN. Again, numerous neurochemicals are implicated, such as glutamate (Biondolillo et al. 2009), GABA (Soderpalm and Berridge 2000), serotonin (De Gobbi et al. 2007), somatostatin (Mantyh and Hunt 1984; Moga and Gray 1985; Panguluri et al. 2009), neurotensin (Moga and Gray 1985), melacin concentrating hormone (Touzani et al. 1993), and corticotropin-releasing factor (De Castro e Silva et al. 2006; Moga and Gray 1985; Panguluri et al. 2009), although their role in mediating centrifugal modulation or even their ability to influence taste-responsive cells remains to be established. To further complicate these descending neural systems recent evidence from our lab indicates that the gustatory regions of NST and PBN receive input largely from separate populations of neurons within the IC, CeA, BNST, and LH (Kang and Lundy 2009). Whether these cell populations express similar or different neurochemicals is unknown. Thus a clear understanding of the true impact that centrifugal regulation of taste processing has on taste-guided behavior awaits experiments that independently manipulate the relevant descending neurochemical pathways.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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