Behavioral/Cognitive

Thalamic Contribution to Cortical Processing of Taste and Expectation

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Taste-related information reaches the gustatory cortex (GC) through two routes: a thalamic and a limbic pathway. While evidence is accumulating on limbic–cortical interactions in taste, very little information is available on the function of the gustatory thalamus in shaping GC activity. Here we rely on behavioral electrophysiological techniques to study taste-evoked activity in GC before and after inactivation of the parvicellular portion of the ventroposteromedial nucleus of thalamus (VPMpc; i.e., the gustatory thalamus). Gustatory stimuli were presented to rats either alone or preceded by an anticipatory cue. The reliance on two different behavioral contexts allowed us to investigate how the VPMpc mediates GC responses to uncued tastants, cued tastants, and anticipatory cues. Inactivation of the thalamus resulted in a dramatic reduction of taste processing in GC. However, responses to anticipatory cues were unaffected by this manipulation. The use of a cue-taste association paradigm also allowed for the identification of two subpopulations of taste-specific neurons: those that responded to gustatory stimulation and to the cue (i.e., cue-and-taste) and those that responded to tastants only (i.e., taste-only). Analyses of these two populations revealed differences in response dynamics and connectivity with the VPMpc.

The results provide novel evidence for the role of VPMpc in shaping GC activity and demonstrate a previously unknown association between responsiveness to behavioral events, temporal dynamics, and thalamic connectivity in GC.

Introduction

The gustatory portion of the insular cortex (also called gustatory cortex; GC) is the primary cortical area responsible for processing taste (Pritchard and Norgren, 2004; Spector and Travers, 2005; Carleton et al., 2010). Electrophysiological analysis of single neuron spiking responses has shown that GC can process physiochemical and psychological aspects of a gustatory experience (Katz et al., 2001; Maffei et al., 2012). Neurons in GC respond to somatosensory stimulation of the oral region (Yamamoto et al., 1981; Katz et al., 2001), chemical identity of the stimulus (Stapleton et al., 2006; Accolla and Carleton, 2008), palatability (Yamamoto et al., 1989; Katz et al., 2001; Accolla and Carleton, 2008; Grossman et al., 2008), and expectation of taste (Stapleton et al., 2006; Saddoris et al., 2009; Samuelsen et al., 2012). These multimodal responses result from the integration of inputs from two pathways (Pfaffmann et al., 1977; Allen et al., 1991): a thalamocortical pathway, which sends input to GC via thalamus (Kosar et al., 1986), and a ventral forebrain pathway, which reaches cortex via a series of limbic areas (Allen et al., 1991; Stone et al., 2011). Despite a growing understanding of the role of the limbic path in conveying affective signals to GC (Grossman et al., 2008; Piette et al., 2012; Samuelsen et al., 2012), no information is available on how thalamocortical inputs contribute to the cortical processing of taste.

The thalamus is universally acknowledged as GC's main source of information about the chemical and physical characteristics of gustatory stimuli (Kosar et al., 1986; Pritchard and Norgren, 2004). Specifically, it is the parvicellular portion of the ventroposteromedial nucleus of thalamus (VPMpc) that plays a fundamental role in processing and relaying gustatory information to GC (Ogawa and Nomura, 1988; Verhagen et al., 2003). Electrophysiological recordings from anesthetized and alert animals have shown that neurons in VPMpc can reliably encode the chemical identity of gustatory stimuli, their temperature, and tactile information from the mouth (Ogawa and Nomura, 1988; Pritchard et al., 1989; Verhagen et al., 2003). Additionally, analysis of the time course of neural responses revealed time-varying modulations of firing rates (Verhagen et al., 2003; Verhagen and Scott, 2004). While a considerable amount of electrophysiological and behavioral work has focused on VPMpc and GC separately, no study to date has directly investigated the contribution of the gustatory thalamus to GC activity.

Here we investigated how single neurons in GC of alert animals respond to taste and expectation of taste before and after temporary inactivation of the VPMpc with the GABA agonist muscimol (MUS). We found that inactivation of the VPMpc altered the general state of GC and dramatically impaired its ability to process gustatory information when tastants were delivered without preceding cues. In a second set of experiments a cue-taste delivery association was established and the effects of thalamic

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inactivation on GC responsiveness to anticipatory cues and cued tastants were explored. This paradigm unveiled responses to classically conditioned cues, which were spared after thalamic suppression. In addition, this paradigm revealed two populations of taste-responsive neurons, i.e., cue-responsive and noncue-responsive, with different sensitivities to thalamic inactivation.

Materials and Methods

Experimental subject
All experimental procedures were performed in accordance with university, state, and federal regulations regarding research animals and were approved by the Institutional Animal Care and Use Committee at Stony Brook University. Female Long–Evans rats (250–350 g) were maintained on a 12 h light/dark cycle with *ad libitum* food and water access, unless otherwise specified.

Surgery
Rats were anesthetized using an intraperitoneally injected ketamine/xylazine/acepromazine mixture (100, 5.2, and 1 mg/kg) with supplemental doses (30% of induction dose) to maintain surgical levels of anesthesia. After placing rats in a stereotaxic device, the scalp was sterilized with an iodine mixture and excised to reveal the skull. Holes were drilled for the placement of anchoring screws, infusion cannulae, and electrode bundles. Microdrivable electrode bundles, consisting of 16 25 μm formvar-coated nichrome microwires, were bilaterally inserted 0.5 mm dorsal to the GC [anteroposterior (AP) 1.4 mm, mediolateral (ML) $\pm$ 5 mm from bregma, dorsoventral (DV) 4 mm from dura]. Once in place, 23 g stainless steel guide cannulae with 30 g stainless-steel wire stylets were bilaterally placed ~500 μm above VPMpc (AP −3.6 mm, ML $\pm$ 1.2 mm from bregma, DV −5.8 mm from dura). All implants were cemented to the skull with dental acrylic. To allow for head restraint, a head bolt was fixed to the rear of the dental acrylic head cap and intra-oral cannulae (IOC) were bilaterally inserted to allow for delivery of gustatory stimuli. Rats were allowed 7–10 d of recovery before beginning behavioral training. Proper placement of electrodes and infusion cannulae was histologically verified using standard procedures (see below and Fig. 1A).

Behavioral Procedures
Uncued taste delivery paradigm. Following recovery from the surgery, rats were placed on a water restriction regimen (45 min of access to water per day) and trained to sit calmly in restraint while receiving deliveries of tastants via a manifold of four fine polyimide tubes into the IOC. Tastants consisted of sucrose (0.1 M), NaCl (0.1 M), citric acid (0.2 M), and quinine (0.001 M). Each trial began with a variable intertrial interval (ITI = 30 $\pm$ 5 s), at the end of which a ~40 μl aliquot of one of four tastants, chosen pseudorandomly, was delivered (pulse duration: ~60 ms). A ~50 μl water rinse followed 5 s after each tastant delivery. After 40 trials (10 of each of the tastes), the session was paused for the bilateral VPMpc intracranial infusions of either 0.2 μl of saline (control) or 0.2 μl of the GABA agonist MUS (100 ng/μl). After removing the stylets, 30ga infusion cannulae, connected to 10 μl Hamilton (Hamilton) syringes via short lengths of PE-10 tubing, were inserted into the guide cannulae. A dual micro-infusion pump (Harvard Apparatus) delivered infusions at a rate of 0.1 μl/min completing the delivery in 2 min. The infusion needle remained for a further 2 min to allow for diffusion away from the infusion site. After this brief infusion period (~7–10 min) the session resumed for another 40 taste deliveries, as above.

Cued taste delivery paradigm. Once rats were habituated to restraint and to receive deliveries of fluid through IOC (as above), a 75 dB, 4 kHz 1 s long auditory tone was introduced. Tastants were delivered immediately after the offset of the tone. Only a single cue was used and at each trial it was associated with the delivery of a taste randomly chosen out of the four available (see above). This design allowed us to investigate the effects of expectation in its most general form, i.e., expectation of the delivery of a gustatory stimulus regardless of its identity, as in Samuelsen et al. (2012). Trials occurred at a 30 $\pm$ 5 s interval. Subjects were exposed to the cue and taste pairing for a minimum of 10 training sessions, each session consisted of at least 80 trials. Rats were considered trained when the cue reliably triggered conditioned mouth movements. Upon verifi-

cation of conditioning, testing procedures initiated. After 40 trials, VPMpc was inactivated following the procedures described above and another 40 trials were performed 7–10 min after removal of the infusion cannula.

Electrophysiological and behavioral data recordings
Signals were recorded using standard multi-electrode techniques (Katz et al., 2001; Samuelsen et al., 2012). Briefly, 32 single-unit channels and 11 continuous local field potentials (LFP) channels were simultaneously amplified, bandpass filtered (at 300—8 kHz for single units and 3–90 Hz for LFP), fed into a multichannel acquisition processor (Plexon), and digitally acquired. Single units were isolated using a template algorithm, cluster-cutting techniques, and examination of interspike interval plots (Offline Sorter, Plexon). Orofacial reactions to tastants and cues were video recorded at 33 frames/s with a camera positioned underneath the mouth of the animal and synchronized with the electrophysiological signals (Cineplex; Plexon).

Analysis of electrophysiological data
Data analysis was performed using Neuroexplorer (Plexon) and custom written scripts in MATLAB (MathWorks).

Analysis of spontaneous activity. For each trial spontaneous LFP were analyzed over 5 s long segments before sensory stimulation (either uncued tastants or cues). Within each recording session power spectral density (PSD) was computed with a ~0.2 Hz resolution and averaged across trials ($n = 40$ for each condition) and electrodes ($n = 11$). LFP signals with movement artifacts were removed from analysis. Pre- and post-infusion PSDs were obtained by averaging across all the sessions for either MUS or saline experiments. For comparison of results before and after infusions power was averaged over two frequency bands (1–9 Hz and 20–50 Hz). A Student's t test was used to compare changes within the same frequency band produced by MUS or saline infusion. To investigate the temporal relationship between LFP and spiking activity, spike-triggered average (STA) of LFP was computed for segments of spontaneous activity. For each hemisphere, spikes from all the single units recorded were combined; the resulting multiunit signal was then used to compute the STA for each LFP channel. STA was calculated over a ± 200 ms window around each spike. Results computed before and after infusion were averaged across LFP channels and hemispheres. To compare the results before and after infusion (i.e., pre-MUS vs post-MUS; pre-saline vs post-saline) the mean STA was computed across the interval from 150 to 200 ms following the spike for each session. Comparisons between STA in different conditions were performed using t tests.

Analysis of single units. For each neuron single trial activity and normalized peristimulus time histograms (PSTHs) were computed for taste and cue presentations before and after infusions. To minimize the effect of baseline changes in firing rates, firing rates within each bin were transformed to z-score (Maren and Hobin, 2007; Ambroggi et al., 2008; Herry et al., 2008; Gutierrez et al., 2010; Erlich et al., 2011; Ghazizadeh et al., 2012) using the following formula: $[(fr_b - fr_{ms})/fr_{sds}]$; where fr_b is the firing rate for each bin, fr_{ms} is the mean spontaneous firing rate during 1 s before the stimulus, and fr_{sds} is the SD of the mean over the same 1 s prestimulus interval. Responses to gustatory stimuli were analyzed over 2.5 s following tastant delivery. Responses to cues were analyzed over the entire 1 s period in which the auditory tone was present. A bin size of 100 ms was used for all analyses, unless otherwise specified in the text. All the analyses were based on statistical comparisons of 10 trials per taste, per condition or 40 trials per cue, per condition.

Taste responsiveness. Neurons were defined as taste selective if two conditions were satisfied: (1) their firing significantly exceeded baseline and (2) they showed significantly different responses to the four tastants. Significance of changes over baseline was established using a t test comparison between baseline bin and evoked bins with correction for familywise error (FWE; Sidak correction or two consecutive significant bins, $p < 0.01$). Neural responses were classified excitatory when firing activity evoked by the tastant significantly exceeded baseline. Responses were classified as inhibitory if taste-evoked activity significantly fell below baseline. Significant difference between responses evoked by the four tastants was determined relying on two-way ANOVA analysis with Sidak

correction for FEW ([taste × time], main effect for taste, $p < 0.05$) for 100 ms bins over a 2.5 s window. Neurons were considered taste selective when they passed the above tests either before or after intrathalamic infusion. Further analyses were performed to establish the time course of responsiveness to one or more tastants (see Fig. 3D) or to address the time course of general taste-selectivity (Fig. 3E). To establish the time course of responsiveness to one or more tastants (Fig. 3D), the number of taste-selective neurons that responded to either one or more tastants was computed for each 100 ms long bin. For each of the four stimuli, significant change from baseline was calculated for each 100 ms bin after stimulus delivery. Within each bin, a neuron was considered responsive to only one tastant (selective) when it showed significant difference from baseline for only one of the four tastants. A neuron was classified as responding to multiple tastants when it exhibited significant changes from baseline for two, three, or all four tastants. Note that neurons responding specifically to one tastant within a bin could be responding to another or to multiple tastants in a different bin. To establish the number of taste-selective neurons for each bin (Fig. 3E), a one-way ANOVA analysis was performed on each 100 ms bin to compare trials for different tastant. Bins with $p < 0.05$ counted as significant, each neuron could contribute to more than one bin. Distributions of number of neurons/ bin before and after thalamic infusion were compared using nonparametric tests (χ^2, $p < 0.05$). The analyses featured in Figure 3, D and E, were performed only on the group of neurons that were taste selective.

Entropy (H). Entropy, a standard measure used in the field to establish the breadth of tuning of neurons, was calculated for each neuron's firing histogram (bin: 500 ms). H value was computed as previously reported (Smith and Travers, 1979) using the following formula:

$$H = -K \sum_{i=1}^{n} Pi \log Pi,$$

where K is a constant and P_i is the proportional response to each tastant. A low H indicates a taste-selective neuron, whereas a high H represents a broadly tuned neuron (therefore a neuron with $H = 0$ responds to a single taste whereas a neuron that responds equally to all tastants would have $H = 1$). To examine changes in tuning following stimulus presentation, H values were averaged across all the neurons for each bin. Poststimulus H values were compared with baseline; bin-to-bin comparisons of H values before and after infusions were performed using a paired t test with correction for type I error (Sidak correction).

Cue responsiveness. Neurons were considered cue responsive if the firing rates evoked by the auditory cue significantly exceeded baseline activity either before or after intrathalamic infusion. Significance was established using t test corrected for FWE (Sidak correction or two consecutive significant bins, $p < 0.01$) to compare binned responses over 1 s with background firing.

Neurons in GC can respond to the sensorimotor component of licking. As in Samuelsen et al. (2012), neurons with strong sensorimotor modulations were removed from the analysis to avoid possible confounds. The PSD was computed in the band between 0.5 and 50 Hz on the basis of a smoothed fast Fourier transform with frequency resolution of ~0.2 Hz. Neurons with a characteristic 6–9 Hz band (licking frequency) were removed (see also Katz et al., 2001 for use of spectral analysis to identify somatosensory neurons).

Comparison of activity before and after infusion. Changes in firing following infusions (i.e., saline and MUS) were assessed for spontaneous activity and evoked responses. For each neuron the significance of the change in spontaneous activity was determined using two-way ANOVAs ([spontaneous × time]) with a conservative p value ($p < 0.01$). Significance of change in taste-evoked activity was assessed using a two-way ANOVA ([stimulus response × time]) with a conservative p value ($p < 0.01$). Comparison between the number of neurons that changed in control condition and those affected by MUS relied on χ^2 test ($p < 0.05$). Also numbers and proportions of neurons significantly responding to cues and taste before and after infusions were compared using χ^2 test ($p < 0.05$).

Comparisons of normalized population PSTHs before and after infusions were performed using paired t test requiring two consecutive bins ($p < 0.01$) to correct for FWE.

Comparisons of subpopulations of neurons. Two populations of neurons with excitatory responses were identified on the basis of their responsiveness to cues and taste (i.e., neurons responding to cues and taste, cue-and-taste, and neurons responding only to taste, taste-only). Visual inspection of population PSTHs (see Fig. 6A) and peak latency analysis of the two behaviorally defined subpopulations suggested a different time course of responses, with taste-and-cue neurons having early peaks and little late activity (average latency to peak: 412 ± 82 ms) and taste-only neurons showing more consistent late peaking, tonic responses (average latency to peak: 1343 ± 92 ms). To statistically compare responses in the two groups of neurons, each neuron's peak z-scores within two time windows representing activity early (0–500 ms) and late (1000–2500 ms) were computed. Peak early and late firing activity was averaged across neurons belonging to the same population. Comparisons between early and late activity, before and after infusions, were performed using t tests ($p < 0.05$).

Mouth movements and orofacial behaviors

Automated frame-by-frame analysis of mouth movements was performed as in Samuelsen et al. (2012). Videos (30 frames/s) of experimental sessions were imported into MATLAB. The image was cropped to isolate the orofacial region and the absolute difference in pixel intensity across consecutive frames was computed. These differences were averaged over the entire cropped image, which provided a single value estimate of mouth movement over a 33 ms period. Segments of videos 1 s before and 3.5 s after each cue were extracted. Only video sessions in which the mouth was not already in motion and a clear, unobstructed view of the cropped image could be obtained were scored. The time course of the difference in intensity was averaged across all cue presentations, before and after MUS infusion. For each session, Student's t tests were performed to compare amplitude of movements averaged across 1 s following cue with background movement averaged over 1 s preceding the cue. Mouth movement records were averaged across all the sessions (pre-infusion only) (see Fig. 4A) and the significance of the movement was established using a confidence interval of three SDs from the baseline. Mouth movements to the cue were used to infer learned classical conditioning. To confirm the validity of the automated analysis, blind frame-by-frame visual inspection was performed on a subset of six randomly chosen cued thalamic inactivation experimental sessions. Latency of mouth movement was assessed as the first visible change in position of the mouth following delivery of the cue and tastant. Difference in latency of mouth movements to cues and taste before and after inactivation were established using t tests.

Histology

Upon completion of experimental sessions, rats were terminally anesthetized, DC current (7 μA) was passed for 7 s through selected wires to mark electrode location, and subjects were transcardially perfused with saline followed by 10% formalin. Fixed brains were stored in 10% formalin and sliced in 40 μm coronal sections. Sections were stained with cresyl violet using standard procedures to verify electrode and cannula placement (Fig. 1A).

Results

To investigate the role of gustatory thalamus in shaping cortical activity, LFPs and ensembles of single units were recorded with movable bundles of multiple electrodes bilaterally implanted in the GC of alert rats (Fig. 1A, left; for schematic of electrode placements). Spontaneous and evoked activity was recorded before and after pharmacological inactivation of VPMpc by local infusion of the GABA agonist MUS (Fig. 1A, right; for schematic of cannula placement). In the first set of experiments rats received uncued presentations of gustatory stimuli through an IOC. After 10 trials of each tastant, taste deliveries were suspended and intrathalamic infusions of either MUS or 0.9% saline (control) were performed. After a ~10 min interval taste deliveries were reinstated and each tastant was presented for a further 10 trials. The second set of experiments relied on similar procedures as the
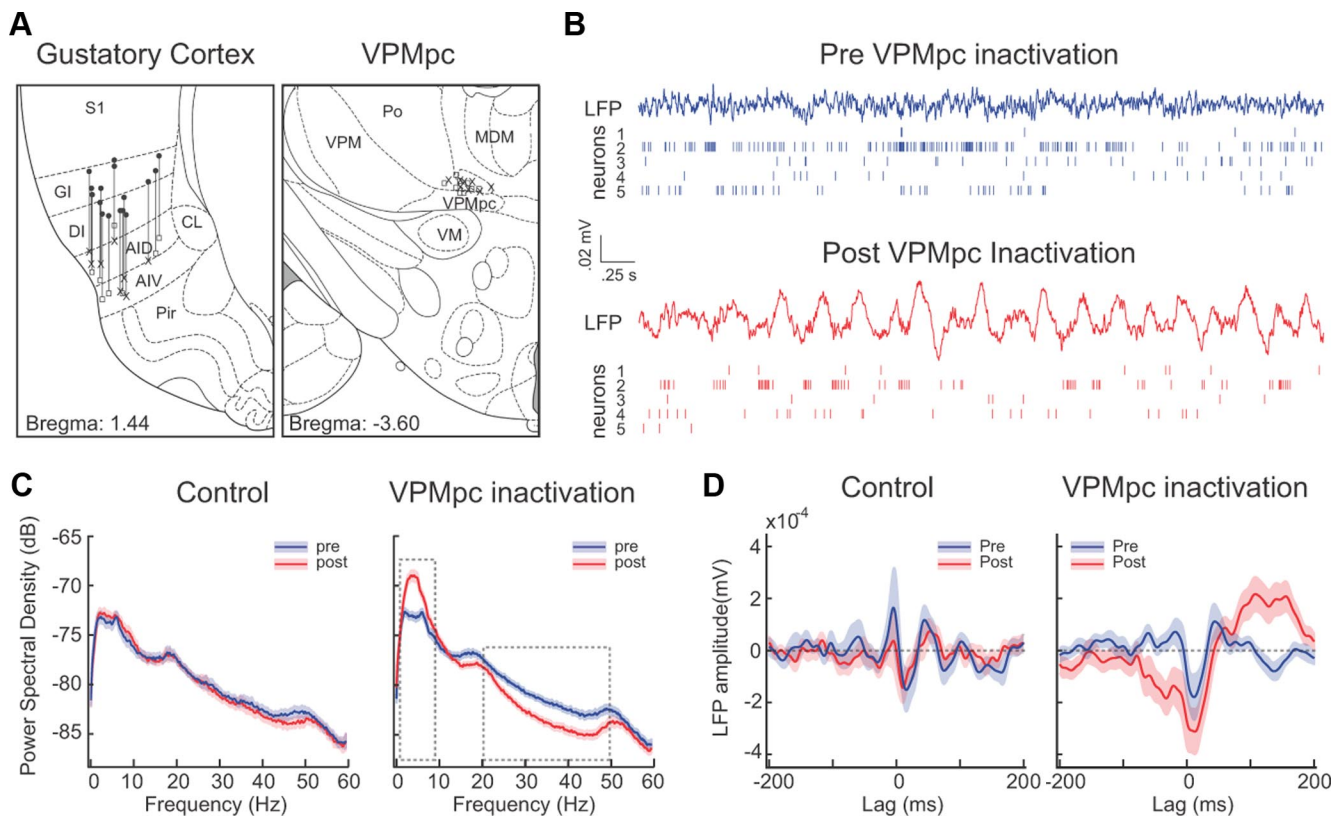
A

Gustatory Cortex   VPMpc

B

Pre VPMpc inactivation

Post VPMpc Inactivation

C

D

Figure 1. Thalamic inactivation changes spontaneous activity in GC. **A**, Schematic representation of coronal sections of the rat brain (reprinted, in part, with permission from Paxinos and Watson, 2007) showing the DV range of recordings in GC (left) and cannulae positioning above gustatory thalamus (VPMpc, right). In the case of electrode placement, the filled circle indicates the first recording site with lines showing the distance to the last recording position (i.e., the most ventral positioning). Xs mark the position in the right and squares the left hemisphere. AID, Agranular insular cortex, dorsal; AIV, agranular insular cortex, ventral; CL, claustrum; DI, dysgranular insular cortex; GI, granular insular cortex; MDM, mediodorsal thalamic nuclei; Pir, piriform cortex; Po, posterior thalamic nuclear group; S1, primary somatosensory cortex; VPM, ventroposteromedial thalamic nuclei; VPMpc, ventroposteromedial thalamic nuclei, parvicellular; VM, ventromedial thalamic nuclei. **B**, Representative traces showing spontaneous LFPs and spiking activity (raster plots below LFP) before (top, blue) and after (bottom, red) MUS infusion. **C**, Average PSD before and after control, saline, infusion (left, $n = 21$), and thalamic inactivation (right, $n = 37$). Thalamic inactivation (right) significantly increased power in the 1–9 Hz band and significantly decreased power in the 20–50 Hz band (dotted boxes, $p < 0.01$). No difference in power was observed for the same bands after control infusion. Blue: pre-infusion; red: post-infusion. Shading around each trace: SEM, **D**, Effect of thalamic inactivation on the spike-triggered average of LFP. The mean value between 150 and 200 ms was significantly affected by MUS infusion (right). No difference was found in the same interval after control infusion (left). Blue: pre-infusion; red: post-infusion.

first, with the notable exception that IOC taste deliveries were made immediately following an associative cue. A total of nine rats (six in the uncued delivery paradigm and three in the cued delivery condition) were investigated for multiple sessions, electrodes were lowered after each recording session to obtain a new ensemble. A total of 251 neurons (179 MUS and 72 control, 34 total sessions) was recorded for the first condition. As for the cued delivery condition, 261 neurons were recorded (165 MUS, 96 control, 24 total sessions).

Thalamic inactivation changes the state of GC
Spontaneous LFPs, as well as population spiking activity, were analyzed to determine the effects of thalamic inactivation on the general state of GC. Figure 1B shows background activity recorded in GC before (blue traces) and after (red traces) intrathalamic infusion of MUS. Visual inspection of the records suggests a clear difference in the temporal patterns, with postinactivation records showing high amplitude, low-frequency oscillations. Power spectral analysis of all the recorded sessions ($n = 37$) confirmed this observation (Fig. 1C). LFPs measurably changed after VPMpc inactivation exhibiting a significantly higher power in the 1–9 Hz band (-73.5 ± 0.3 dB before and -71.1 ± 0.3 dB after MUS, $p < 0.01$, $n = 37$ sessions) and significantly lower power in 20–50 Hz band (-81.2 ± 0.3 dB before and -83.0 ± 0.3 dB after

MUS, $p < 0.01$, $n = 37$). No significant difference was observed for the same bands after infusion of saline into VPMpc (1–9 Hz band: -74.0 ± 0.5 dB before and -73.5 ± 0.4 dB after saline, $p > 0.1$, $n = 21$; 20–50 Hz band: -81.3 ± 0.4 dB before and -81.8 ± 0.4 dB after saline, $p > 0.1$, $n = 21$). To investigate whether spectral changes in LFP were associated with modulations of firing patterns, the STA of LFP was computed on the basis of multiunit firing for each session. Figure 1D shows significant changes in the STA after thalamic inactivation. The average peak in the 150–200 ms interval after 0 lag went from $-1*10^{-5} \pm 2*10^{-5}$ mV before inactivation to $1*10^{-4} \pm 3*10^{-5}$ mV following MUS ($p < 0.01$, $n = 37$) (Fig. 1D, right). Spikes were associated with the larger deflections of LFPs, suggesting an increase in the spike-field correlation, which paralleled the changes in LFP rhythmicity. The representative traces in the bottom of Figure 1B indicate the correlation between firing in GC neurons and LFP oscillations. No such changes were observed for the 150–200 ms interval in the case of saline infusions ($-3*10^{-5} \pm 2*10^{-5}$ before and $3*10^{-6} \pm 2*10^{-5}$ after saline, $p > 0.1$, $n = 21$) (Fig. 1D, left). In addition to the enhancement in slow rhythmicity, thalamic inactivation was also associated with changes in spontaneous firing rates. Following infusion of MUS, 247 of the 344 (71.8%) neurons showed significant differences in spontaneous firing rates; 49.8% (123/247) of these increased their firing frequency, while
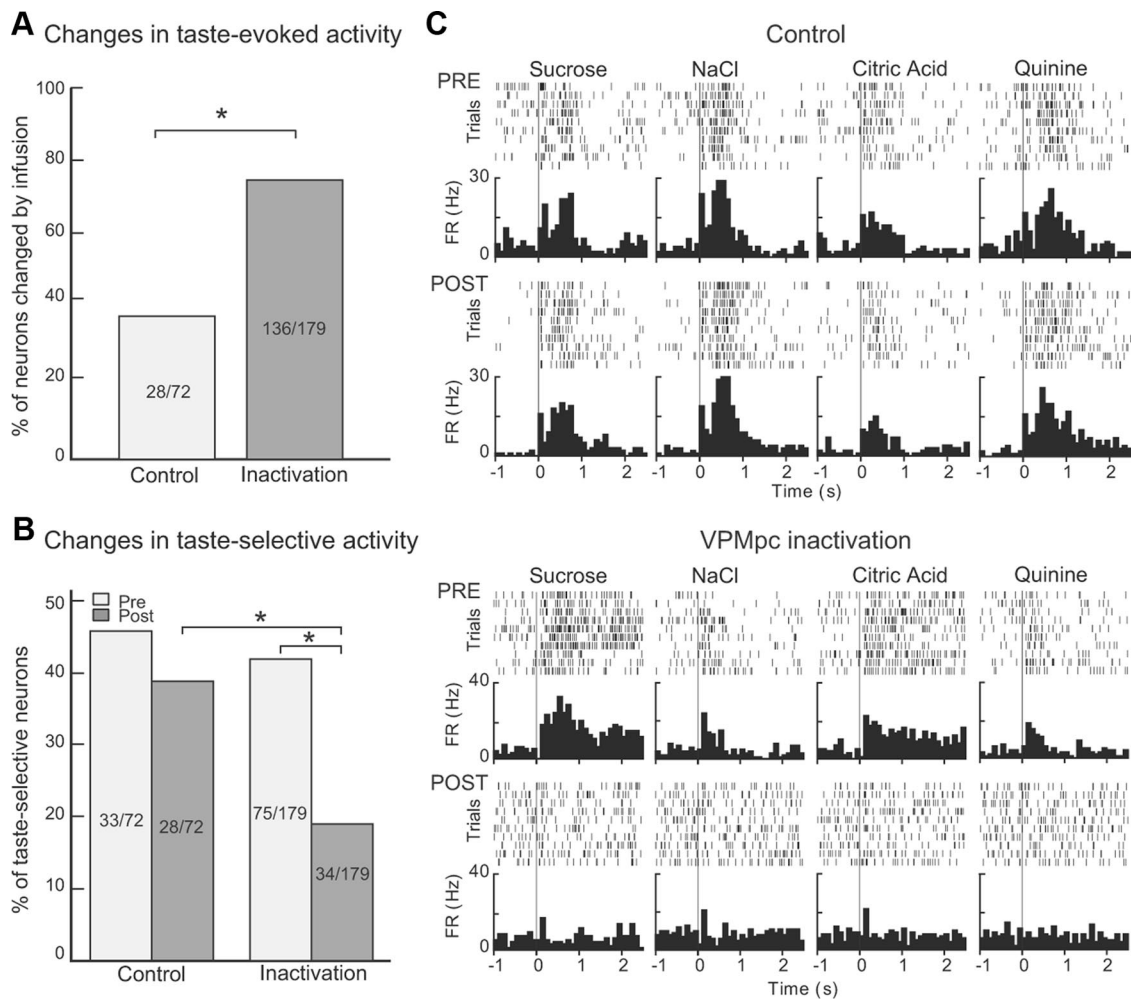
Figure 2. Impact of VPMpc inactivation on spiking activity evoked by uncued tastants. *A*, Histogram showing the proportion of neurons whose taste response was significantly affected by infusions. Light gray bar, saline infusion; dark gray bar, MUS. The proportion of neurons that exhibited a significant difference in taste-evoked firing rates was significantly greater (*$p < 0.01$) after MUS infusion. *B*, Histogram showing the proportion of taste-specific neurons before and after thalamic infusions. Left pair, control infusions; right, MUS inactivation. Light gray, before infusion; dark gray, after infusion. Thalamic inactivation resulted in a significant reduction of the number of taste-selective neurons relative to pre-infusion and relative to post-saline infusion (*$p < 0.01$). *C*, Representative raster plots and PSTHs of two taste-selective neurons responding to the four tastants (Sucrose, NaCl, Citric Acid, and Quinine). Top, Taste responses of a representative neuron before (top) and after (bottom) control infusion. Bottom, Activity of another neuron before and after VPMpc inactivation. Control infusions resulted in little change of activity, while MUS infusion dramatically depressed spiking activity.

50.2% (124/247) decreased. These results show that inactivation of VPMpc alters the state of GC, a result consistent with previous observations of other cortical areas.

VPMpc inactivation alters single neuron responses to uncued gustatory stimulation

To determine how VPMpc inactivation impacted evoked activity, we recorded single neuron spiking responses to gustatory stimuli before and after intrathalamic infusion of MUS. Two-way ANOVA ([time × condition]) analysis of firing responses revealed that 76.0% (136/179) of the neurons had a significant change in responsiveness after VPMpc inactivation (Fig. 2A). Following saline infusion, 38.9% (28/72) of neurons changed their responses, a time-dependent change similar to that observed across sessions with analogous duration (Fontanini and Katz, 2006). The percentage of neurons changing their responses following saline was half of that observed for MUS and the difference between the two conditions was significant ($p < 0.01$). To further assess the impact of inactivation on gustatory processing, we computed the proportion of neurons that produced taste-

selective responses in the first 2.5 s following stimulation. The result of this analysis is described in Figure 2B. Before thalamic inactivation, 41.9% (75/179) of the neurons recorded were taste selective, a result consistent with prior studies (Fontanini and Katz, 2006; Piette et al., 2012). As expected, the changes of firing patterns observed for saline infusions did not result in any significant change of the proportion of taste-selective neurons (before 33/72, 45.8%; after 28/72, 38.9%, $p > 0.1$), nor in the number of neurons responsive to individual tastants (Sucrose: before 20/72, 27.8%; after 21/72, 29.2%, $p > 0.1$; NaCl: before 18/72, 25.0%; after 18/72, 25.0%, $p > 0.1$; Citric Acid: before 25/72, 34.7%; after 22/72, 30.6%, $p > 0.1$; Quinine: before 22/72, 30.6%; after 20/72, 27.8%, $p > 0.1$.). However, VPMpc inactivation lead to a dramatic reduction in the number of taste-selective neurons (from 75/179, 41.9%, before inactivation to 34/179, 19.0%, after, $p < 0.01$). The absolute number of single neurons responding to each individual tastant was also significantly reduced by VPMpc inactivation (Sucrose: before 38/179, 21.2%; after 19/179, 10.6%, $p < 0.01$; NaCl: before 44/179, 24.6%; after 13/179, 7.3%, $p < 0.01$; Citric Acid: before 51/179, 28.5%; after 20/179, 11.2%, $p < 0.01$;

Quinine: before 46/179, 25.7%; after 18/179, 10.1%, $p < 0.01$). Interestingly, VPMpc inactivation only marginally influenced the relative distribution of neurons that responded to individual tastants within each group of taste-selective cells; of the 75 neurons that responded to taste before MUS, 50.6% (38/75) responded to Sucrose, 58.6% (44/75) to NaCl, 68% (51/75) to Citric Acid, and 61.3% (46/75) to Quinine. Of the 34 neurons that were taste-selective after thalamic inactivation, 55.8% (19/34) responded to Sucrose, 38.2% (13/34) to NaCl, 58.8% (20/34) to Citric Acid, and 52.9% (18/34) to Quinine. The raster plots and PSTHs in Figure 2C illustrate examples of two taste-selective neurons responding to Sucrose, NaCl, Citric Acid, and Quinine before and after infusions of MUS or saline.

To determine whether the modification of evoked firing rates was a general phenomenon among taste-selective neurons and to assess the effects of inactivation on the time course of firing responses, we computed the normalized population PSTH (z-score) before and after thalamic inactivation. Taste-selective neurons were divided into two groups: those that had an inhibitory response to taste stimulation (MUS: $n = 15$; control: $n = 9$) and those that responded with excitation (MUS $n = 68$; control: $n = 28$). Figure 3, A and B, show the result of the analysis of inhibitory and excitatory responses, respectively. Normalized population PSTHs show that inactivation results in dramatic modification of the time course of evoked activity in both groups. Figure 3A features the normalized population PSTHs (z-scores averaged across cells) for neurons with an inhibitory response to taste stimulation before and after saline (left) and MUS infusion (middle). While saline infusion had no effect on cells that responded with inhibition, MUS infusion completely abolished the response. The z-score of the response averaged in the first second following taste presentation went from -3.94 ± 0.82 to -0.54 ± 0.26 ($p < 0.01$, $n = 15$). Figure 3A, right, details the effects of VPMpc inactivation on average inhibitory responses to individual tastants (top) and representative raster plot and PSTH for the effects of MUS on an inhibited neuron (bottom). As with inhibited neurons, saline infusion had no effect on neurons that responded with excitation (Fig. 3B, left). However, VPMpc inactivation led to a dramatic reduction of taste responses, as shown in Figure 3B (middle: population PSTH; right, top: average population responses to all the tastants; right, bottom: representative excitatory neuron raster plot and PSTH). While no significant change was observed in the first 300 ms after taste stimulation (from 4.07 ± 1.42 before to 1.84 ± 0.77 after MUS, $p > 0.05$, $n = 68$), the difference became significant for the next ~ 2 s of the response (Fig. 3B, middle, black bar), when an average z-score of 2.90 ± 0.63 before inactivation decreased to 0.64 ± 0.20 after ($p < 0.01$, $n = 68$). Additional analysis of the responsiveness in the first 300 ms was performed to determine whether the residual early (i.e., <300 ms) activity contained taste-related information. Before inactivation, 31 of 83 taste-specific neurons showed significant increases in firing in the first 300 ms following taste delivery. Of this group of 31 neurons, 16 produced taste-specific responses within this interval. These numbers were significantly reduced by thalamic inactivation. After thalamic inactivation only 16 neurons showed increases in firing activity during the first 300 ms ($p < 0.01$), and only three were taste selective ($p < 0.01$). These results indicate that while VPMpc inactivation partially spared early increases in firing activity, early taste processing was depressed.

To more extensively evaluate how thalamic inactivation impacted the time course of taste processing, modifications in taste selectivity were computed for the 2.5 s following the presentation of the stimulus. To evaluate how thalamic inactivation changed the tuning for single neurons over time, the entropy value (H value), a standard measure used in taste research (Smith and Travers, 1979), was measured. H value was computed for successive 500 ms bins following stimulus delivery. Before inactivation, entropy dropped significantly 500 ms following taste presentation (spontaneous H value: 0.91 ± 0.01, H value between 0.5 and 1 s: 0.84 ± 0.02, $p < 0.01$, $n = 83$), indicating an increase in taste selectivity, and remained significantly lower for the entire 2.5 s interval. After thalamic inactivation, taste-selective neurons exhibited no decrease in H value relative to baseline (spontaneous H value: 0.92 ± 0.01, H value between 0.5 and 1 s: 0.89 ± 0.01, $p > 0.1$, $n = 83$). Such a lack in taste-evoked reduction of H value could indicate a broadening of the tuning induced by thalamic inactivation or a flat-out disruption of taste specificity >2.5 s following gustatory stimulation.

To disambiguate the interpretation of the H value result, a second analysis of taste tuning was performed. For each 100 ms bin, the number of neurons that responded either to only one or to more than one tastant was computed. Figure 3D shows the bin-by-bin count of neurons responsive to only one taste (solid blue line, dashed red line) or selective to more stimuli (solid light blue line, dashed pink line), before (solid lines) or after (dashed lines) MUS. Before VPMpc inactivation, both narrowly and broadly tuned responses could be observed for each bin. The time course of selective and broad responses was significantly different ($p < 0.05$). Responses to multiple tastants peaked in the first 500 ms and decreased afterward; whereas the count of responses selective for only one tastant peaked after 1 s. After thalamic inactivation, the predominant effect was a significant reduction of the counts for both single-tastant selective neurons ($p < 0.01$) and broadly tuned neurons ($p < 0.01$). Indeed, rather than seeing an increase in the number of broadly tuned neurons following thalamic inactivation, we observed a general reduction of taste processing. To further validate the interpretation that the lack in taste-evoked reduction of H value following MUS indicates a disruption of taste processing, we computed how many of the taste-selective neurons exhibited taste selectivity for each 100 ms time bin of the 2.5 s time course. In normal conditions the highest number of taste-selective neurons (36.1%, 30/83) occurred at 1 s after taste onset, remaining elevated for the entire time period. After inactivation the distribution was significantly decreased ($p < 0.01$), and showed sparser responsiveness across the 2.5 s, with only a few neurons being taste selective (Fig. 3C).

Overall these experiments demonstrate that VPMpc inactivation greatly reduces GC responsiveness to uncued gustatory stimuli delivered via IOC. Excitatory taste-selective neurons can still elevate their firing rates in response to stimulation in the first 300 ms following taste delivery; however, firing rates are dramatically affected after that time.

Responses to anticipatory cues survive thalamic inactivation
Recent results show that the responsiveness of GC to taste is influenced by expectation and that gustatory cortical neurons respond to cues anticipating the availability of taste (Samuelsen et al., 2012). To study the effects of VPMpc inactivation on expectation-related activity, rats were conditioned to associate an auditory cue with the delivery of tastants. Figure 4A shows the result of an automated frame-by-frame video analysis of mouth movements establishing learning of the association (see Materials and Methods). The time course of mouth movements averaged across all the sessions showed a significant increase in activity following the anticipatory cue ($p < 0.01$, see bar below the trace). Trial-by-trial analysis of video records
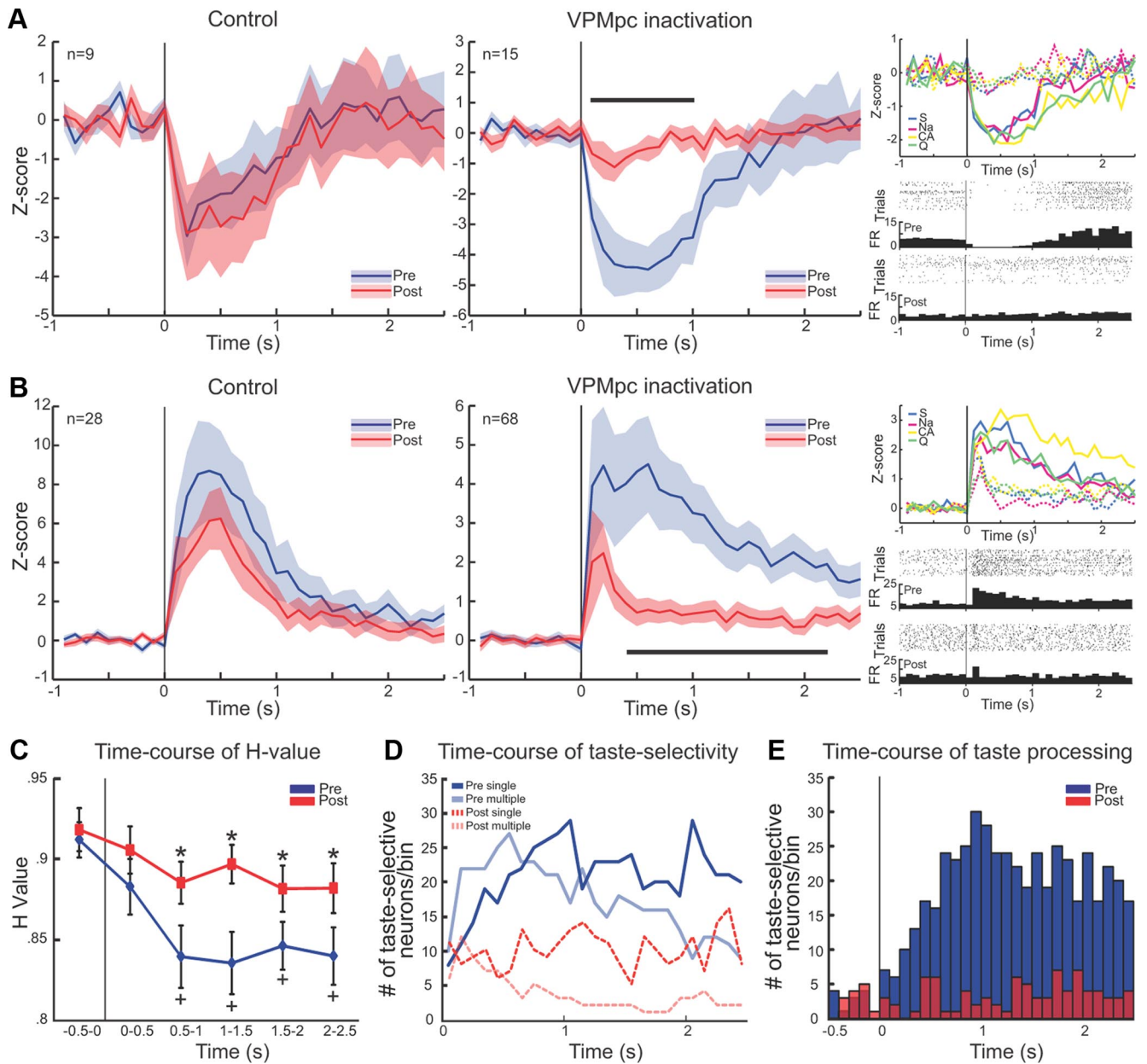
Figure 3. Thalamic inactivation alters the time course of responses to uncued taste. **A**, Normalized population PSTHs (i.e., z-score) of taste-selective neurons inhibited by uncued taste stimulation ($t = 0$, vertical line) before and after saline (control, left) and MUS (middle) infusions. The shaded area represents the SEM. No significant difference was detected in taste responses before (blue trace) or after (red trace) control infusion ($n = 9$). MUS infusion ($n = 15$) resulted in a significant alteration in taste response. The black horizontal line indicates the time points for which the difference between pre-infusion and post-infusion z-scores is significant ($p < 0.01$). Top right, Normalized population PSTH of each tastant (S: sucrose, blue; Na: NaCl, magenta; CA: citric acid, yellow; Q: quinine, green) before (solid lines) and after (hashed lines) thalamic inactivation. Responses to individual tastants are inhibited as the average responses show (left and middle). Bottom right, Representative raster plot and PSTH of a neuron inhibited by taste stimulation before (top) and after (bottom) thalamic inactivation. Note the complete disappearance of the inhibitory response. **B**, Normalized population PSTHs (i.e., z-score) of taste-selective neurons excited by uncued taste stimulation. Control infusion (left) ($n = 28$) did not significantly modify the time course of the response of taste-selective neurons. MUS infusion (middle; $n = 68$) resulted in a severe modification of time course of the taste response (black horizontal line indicates the time at which responses are significantly different, $p < 0.01$). While part of the early component of the response is spared, responses after 300 ms were almost completely suppressed. Top right, Normalized population PSTH of each tastant, each showing similar early responses with late suppression after thalamic inactivation. Bottom right, Raster and PSTH for a representative excitatory response. **C**, Time course of H value following gustatory stimulation before (blue line) and after (red line) MUS. Gustatory stimulation resulted in a significant reduction of the H value relative to baseline (see $^+p < 0.01$, after 500 ms). No significant drop in H value was observed for any time bin after VPMpc inactivation. The time course of H values after MUS was significantly different from that pre-inactivation ($*p < 0.01$). **D**, Time course of taste selectivity measured as the number of taste-selective neurons responding selectively to one (solid dark blue and dashed red) or to more than one (solid light blue and dashed pink) tastant within each time bin. Solid blue traces represent data before MUS; dashed traces represent selectivity after MUS. VPMpc inactivation significantly ($p < 0.01$) reduced the number of neurons responding to one or more tastants. Note that neurons responding specifically to one tastant within a bin could be responding to another or to multiple tastants in a different bin. **E**, The number of neurons that exhibited taste selectivity during each successive 100 ms bin following taste presentation was significantly reduced by thalamic inactivation ($p < 0.01$). Blue bars, Before inactivation; red bars, after MUS.

confirmed the significance of cue-evoked movement for each individual session (Fig. 4A, inset). Figure 4B shows a quantification of the latency of mouth movements. This analysis revealed that cue-triggered movements occur at a longer latency than taste-evoked

movements (255 ± 9 ms for cue vs 158 ± 3 ms for taste, $p < 0.01$, $n = 6$) and that thalamic inactivation significantly delayed the onset of movement to cue (314 ± 12 ms, $p < 0.01$, $n = 6$) and to taste (254 ± 24 ms, $p < 0.01$, $n = 6$).

Cue-evoked neural activity was first assessed by quantifying the number of neurons whose firing was significantly modulated by the cue. Consistent with prior results (Samuelsen et al., 2012), we found that cues can reliably evoke anticipatory responses in GC neurons (71/261, 27.2%). Figure 5A shows that neither saline (Pre-saline: 20.8%, 20/96; Post-saline: 18.8%, 18/96; $p > 0.1$) nor MUS (Pre-inactivation: 22.4%, 37/165; Post-inactivation: 18.2%, 30/165; $p > 0.1$) infusions significantly reduced the number of cue-responsive neurons. The persistence of cue responsiveness after thalamic inactivation was confirmed by analysis of population PSTHs of neurons responding to the cue either before or after MUS. Analysis of the population PSTH of neurons whose firing rates were significantly modulated by the cue (Fig. 5B) revealed that VPMpc inactivation does not significantly inhibit cue responses. Indeed, paired t test analysis of the 1 s response to the cue before and after MUS revealed no significant difference ($p > 0.1$). Peak of average z-scores before MUS was 6.04 ± 1.14, after MUS 4.60 ± 0.62 ($p > 0.1$, $n = 47$). Average z-score over the entire second went from 1.96 ± 0.75 before infusion to 1.18 ± 0.37 after ($p > 0.1$, $n = 47$). Although visual inspection might suggest a trending reduction or shift in responses to cue, no significant changes were observed after VPMpc inactivation. The raster and PSTH detailed on the right show a representative neuron that maintains its responsiveness after VPMpc inactivation. Not surprisingly, analysis of saline infusions revealed no significant change in the normalized population PSTH of cue-responsive neurons. Average z-score over the entire second went from 1.73 ± 0.86 before saline infusion to 2.02 ± 0.89 after ($p > 0.1$, $n = 24$). Also the average peak was not affected by saline infusion (before saline: 5.11 ± 1.09, after saline: 5.84 ± 1.42, $p > 0.1$, $n = 24$). Altogether these results suggest that, even after VPMpc inactivation, information about anticipatory cues effectively reaches GC and activates neurons.

Subpopulations of neurons are differentially affected by VPMpc inactivation

Taste responsiveness in GC is known to be influenced by general expectation; yet the contribution of the thalamus to responses during this behavioral state is unknown. To address this issue, spiking activity evoked by cued gustatory stimulation was analyzed before and after intrathalamic infusion of MUS. As with the previous experiments relying on uncued gustatory stimulation, comparison of responses before and after MUS infusion revealed that VPMpc inactivation modified taste responsiveness in a proportion of neurons significantly larger than saline infusions (saline: 42.7%, 41/96; MUS: 61.2%, 101/165; $p < 0.01$). After thalamic inactivation, the number of taste-selective neurons was significantly reduced compared with pre-inactivation (before MUS: 32.7%, 54/165; after: 14.5%, 24/165, $p < 0.01$) and control infusion (before saline: 29.2%, 28/96, $p < 0.01$; after: 25.0%, 24/96, $p < 0.01$).

To further understand the effects of thalamic inactivation on responses to cued tastants, neurons with taste-selective excitatory
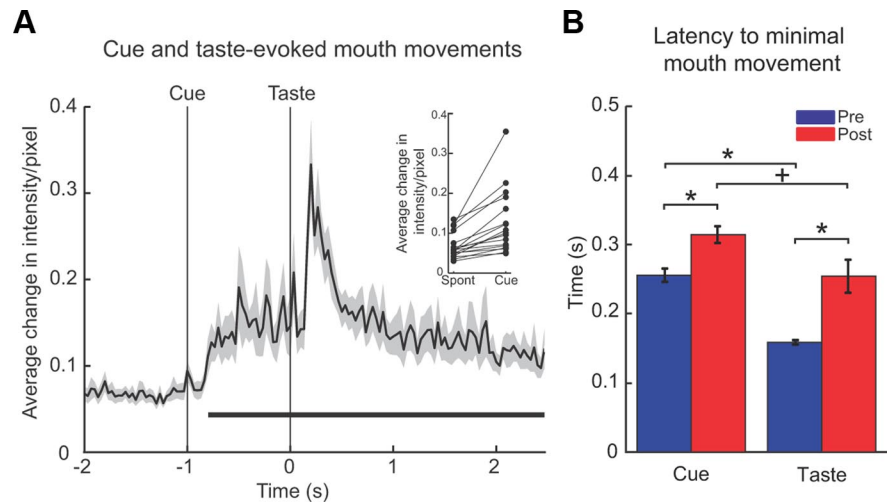
Figure 4. Mouth movements evoked by anticipatory cues and cued tastants. **A**, Shows the average time course of mouth movements as detected by automatic frame-by-frame video analysis. The shading corresponds to the SEM. The thick bar below the trace indicates the time points at which mouth movements are significantly different from baseline. The significant increase in mouth movements occurred quickly after cue onset (vertical line at -1 s) and continued well after taste delivery (vertical line at 0 s). The inset shows the average movement (indexed as change in pixel intensity) before the onset of the cue and during the cue for each session. Cue-evoked movements were significant in all the sessions analyzed. **B**, Average latency of the minimal mouth movement to cue (left) and taste (right) stimulation before (blue bars) and after (red bars) thalamic inactivation. Latency to cue and taste before thalamic inactivation is significantly shorter than after thalamic inactivation (cue, before MUS: 255 ± 10 ms, after MUS: 314 ± 12 ms, $p < 0.01$, $n = 6$; taste, before MUS: 158 ± 3 ms, after MUS: 254 ± 24, $p < 0.01$, $n = 6$). Minimal mouth movement latency was significantly slower to cues compared with taste delivery regardless of condition. *$p < 0.01$, +$p < 0.05$.

responses were divided into two subpopulations on the basis of their cue responsiveness: those activated by both cue and cued taste (from here on cue-and-taste neurons) and those responding to cued taste, but with no significant response to the anticipatory cue itself (from here on taste-only neurons). Figure 6 details the normalized population PSTHs (Fig. 6A) and representative examples (Fig. 6B, Fig. 7A2,B2, top, for more examples) for cue-and-taste and taste-only neurons showing excitatory responses to taste. Comparison of the time course of the responses to gustatory stimulation before thalamic inactivation revealed significantly different response profiles for the two groups ($p < 0.01$, $n = 25$ and $n = 40$). Cue-and-taste neurons ($n = 25$) displayed firing modulations that peaked early (average time of the peak: 412 ± 82 ms) and that returned close to baseline after 1 s following gustatory stimulation (Fig. 6B, left, Fig. 7A2, representative neurons). Taste-only neurons ($n = 40$), had an average time of the peak at 1343 ± 92 ms, a value significantly different from that of cue-and-taste neurons ($p < 0.01$). As a group, taste-only neurons showed little tendency to phasic early firing. Regardless of the presence of an early elevation in firing rates, which could be observed for some neurons (Fig. 6B, right, Fig. 7B2, left), activity in taste-only neurons tended to remain tonically protracted well after 1 s (Fig. 7B2, left, representative neuron). Taste-only neurons had patterns of late activity that were significantly higher than those observed for cue-and-taste neurons. Figure 6C details a quantification of the firing patters in the two populations. For cue-and-taste neurons (Fig. 6C, left, dark gray squares; right panel, dark gray bars), average peak activity was significantly higher in the first 0.5 s following tastant delivery than in the later part of the response, i.e., from 1 to 2.5 s after stimulus (z-score early:12.87 ± 2.69; z-score late:4.34 ± 0.87, $p < 0.01$, $n = 25$). Taste-only neurons (Fig. 6C, middle, light gray squares; right, light gray bars), on the other hand, had late peak responses that were at least as strong as early ones (z-score early: 6.00 ± 3.77, z-score late:6.99 ± 1.19, $p > 0.1$, $n = 40$). Visual inspection of the
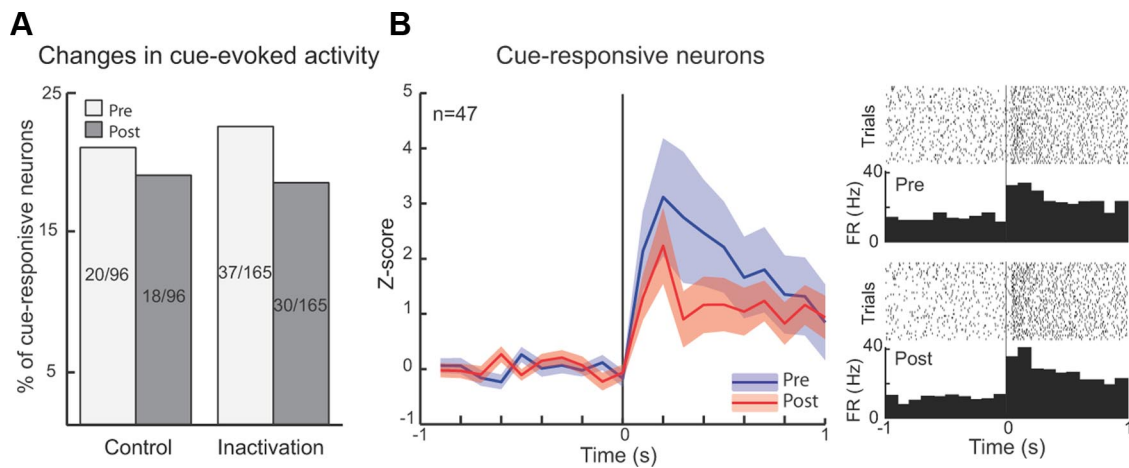
Figure 5. Effects of VPMpc inactivation on responses to anticipatory cues. ***A***, Proportion of cue-responsive neurons before (light gray) and after (dark gray) control (left bars) and MUS (right bars) infusions. The percentage of cue-responsive neurons was not significantly altered by thalamic inactivation ($p > 0.1$). The effects of MUS were not significantly different from those of saline infusions, $p > 0.1$). ***B***, Left, Normalized population PSTH (z-score, $n = 47$) of cue responses before (blue trace) and after (red trace) MUS infusion. The onset of the cue occurs at 0 s. Shading represents SEM. No significant difference was detected in cue responses following MUS. Right, Details raster plots and PSTHs of a representative cue-responsive neuron. Top, Before MUS; bottom panel, after MUS.

scatter plot suggested a bias toward small responses; however, all the neurons showed taste responses that were significant. Direct comparison of the average peak activity in the 0–0.5 s and 1.0–2.5 s time windows for the two populations revealed no significant difference early (z-score for cue-and-taste: 12.87 ± 2.69; z-score for taste-only: 6.00 ± 3.77, $p > 0.1$) but a significantly higher late activity for taste-only neurons (z-score for cue-and-taste: 4.34 ± 0.87; z-score for taste-only: 6.99 ± 1.19, $p < 0.05$).

The two populations of neurons differed not only in their taste response dynamics, but also in their susceptibility to VPMpc inactivation (Fig. 7). As Figure 7,*A1* and *A2*, show, the activity increase upon stimulus delivery for cue-and-taste neurons did not exhibit a significant change at either time point after MUS infusion. Comparison of the normalized population PSTH (Fig. 7*A1*) before (blue) and after (red) inactivation revealed no significant difference in the response to taste. Figure 7*A2* details a representative cue-and-taste neuron maintaining its responsiveness after MUS. Quantification of early and late peak activity (Fig. 7*C*, left and right) further confirmed this result. The same pattern of an early increase in activity following cued taste was still observed after thalamic inactivation. Indeed, as Figure 7*C* shows, no significant reduction was observed in activity either early (z-score early: 13.87 ± 4.17 vs 9.23 ± 2.28, $p > 0.1$, $n = 14$) or late (z-score late: 4.25 ± 1.22 vs 3.47 ± 0.90, $p > 0.1$, $n = 14$) following MUS. Control experiments revealed that intrathalamic infusions of saline had no affect on cue-and-taste peak activity either early (z-score before infusion: 11.61 ± 3.25; z-score after 9.51 ± 3.63, $p > 0.1$, $n = 11$) or late (z-score before infusion: 4.45 ± 1.27; z-score after 4.04 ± 0.57, $p > 0.1$, $n = 11$).

Opposite to cue-and-taste, responses to cued stimuli in taste-only neurons were almost completely abolished by MUS infusion. Figure 7, *B1* and *B2*, show population PSTHs and representative examples for taste-only neurons' activity before and after inactivation of VPMpc. Population PSTHs were significantly ($p < 0.01$, solid lines above traces in Fig. 7*B1*) reduced by intrathalamic infusion of MUS. A comparison of average peak activity before and after MUS (Fig. 7*C*, middle and right, taste-only) revealed that the average peak activity in the late (1–2.5 s) period was significantly reduced by VPMpc inactivation (z-score before MUS, blue squares and bars: 7.84 ± 1.68; z-score after MUS, red circles and bars: 3.17 ± 0.48, $p < 0.01$, $n = 27$). In this

population MUS also reduced peak activity in the early period (0–0.5 s), from a z-score of 8.19 ± 5.56 to a z-score of 2.53 ± 0.50 after MUS ($n = 27$, $p > 0.1$), even though the variability in the occurrence of the early onset in taste-only neurons prevented significance. The reduction of responsiveness in taste-only neurons was not an effect of infusion, as saline infusion did not significantly impact responses in either the early peak (z-score before infusion: -1.46 ± 0.70; z-score after 0.58 ± 0.82, $p > 0.1$, $n = 13$) or late peak (z-score before infusion: 5.21 ± 1.06; z-score after 4.15 ± 1.14, $p > 0.1$ $n = 13$) period.

Additional analyses were performed to establish how VPMpc inactivation affected taste processing in the two populations of neurons. Regardless of the magnitude of their responses, all the neurons in either group were taste selective before inactivation. The distribution of taste selectivity was investigated for both cue-and-taste and taste-only neurons before and after MUS. Before inactivation out of the 14 cue-and-taste neurons 0 were selective to one tastant, 3 to two, 4 to three, and 7 to all four. After thalamic inactivation 2 neurons were selective to one tastant, 2 to two, 1 to three, and 5 to all four. As a group only 30.7% of cue-and-taste neurons (4/13) lost taste selectivity after inactivation. On the contrary, in the case of taste-only neurons, 66.6% (18/27) of the cells lost taste selectivity. Specifically, before VPMpc inactivation, out of 27 taste-only units, 3 were selective to one taste, 13 to two, 7 to three, and 4 to all four. After inactivation 5 neurons responded only to one tastant, 4 to two, 0 to three, and 0 to all four.

Altogether, these results show that the effects of VPMpc inactivation on taste-evoked activity vary depending on whether taste coding neurons respond to an anticipatory cue or not.

Discussion

The results presented here unveil how neural activity in gustatory cortical circuits is driven by thalamic inputs. Consistent with the role of the thalamus in other sensory systems (Steriade, 2000; Huguenard and McCormick, 2007; Poulet et al., 2012), we found that VPMpc contributes to establish the background state of GC. Analysis of LFP and multi-unit activity revealed an increase in low-frequency oscillations and a reduction in high-frequency activity following silencing of VPMpc with the GABA agonist MUS. Not surprisingly, inactivation of the thalamus also influenced the ability of GC to process gustatory information. In a first set of

experiments, gustatory stimuli were delivered through IOC at ~30 s intervals. Single neuron responses to uncued gustatory stimulation were dramatically modified by thalamic inactivation. While we did observe baseline changes in single neuron firing rates also for control infusions, a result consistent with time-dependent modifications in the state of GC networks (Fontanini and Katz, 2006), MUS infusions modified activity in twice as many neurons. Such a MUS-dependent change in activity translated into a dramatic reduction of the number of taste-selective neurons (a phenomenon not observed for control infusions). In taste-selective neurons, we observed a disappearance of inhibitory responses and a marked decrease of excitatory responses to taste. Only an early and short-lasting elevation in firing rates survived after MUS infusion. In control conditions, early activity evoked by uncued gustatory stimuli contains relatively little taste-related information (Katz et al., 2002; Samuelsen et al., 2012), thalamic inactivation results in an additional reduction of early gustatory processing. Previous reports suggested that early activity reflects somatosensory information (Katz et al., 2001), recent data from cued deliveries of tastants also propose that early activity might be modulated by top-down expectational signals (Samuelsen et al., 2012). While our results do not allow us to identify the specific source of early elevations in firing rates, their survival after VPMpc inactivation points to the role of extra-VPMpc inputs in driving short-latency elevations in firing rates.

To further investigate the role of the thalamus in the context of an expectation-related paradigm, a second set of experiments was performed. In these experiments tastants were presented following an anticipatory cue. This experimental configuration allowed us to identify responses to anticipatory auditory cues in GC. Cue responsiveness was not influenced by thalamic inactivation; indeed, while we saw a minor trend toward decreased cue responsiveness, it never reached significance. The second set of experiments also unveiled two groups of neurons showing excitatory taste-selective responses: those that responded to taste and cue and those that responded only to taste. The two groups differed not only in their behavioral specialization, but also in the overall time course of their responses to gustatory stimulation. While neuron-by-neuron inspection of taste responses revealed within-group variability, analysis of average responses showed that taste-and-cue neurons tended to respond with early and short-lasting elevations in firing rates, whereas taste-only neurons showed tonic firing. Importantly the two populations dramatically differed also in their susceptibility to thalamic inactivation. MUS in VPMpc resulted in the disappearance of responses from taste-only neurons, while taste-evoked elevations in firing rates neurons were relatively spared by thalamic inactivation in taste-and-cue neurons. Taste selectivity was
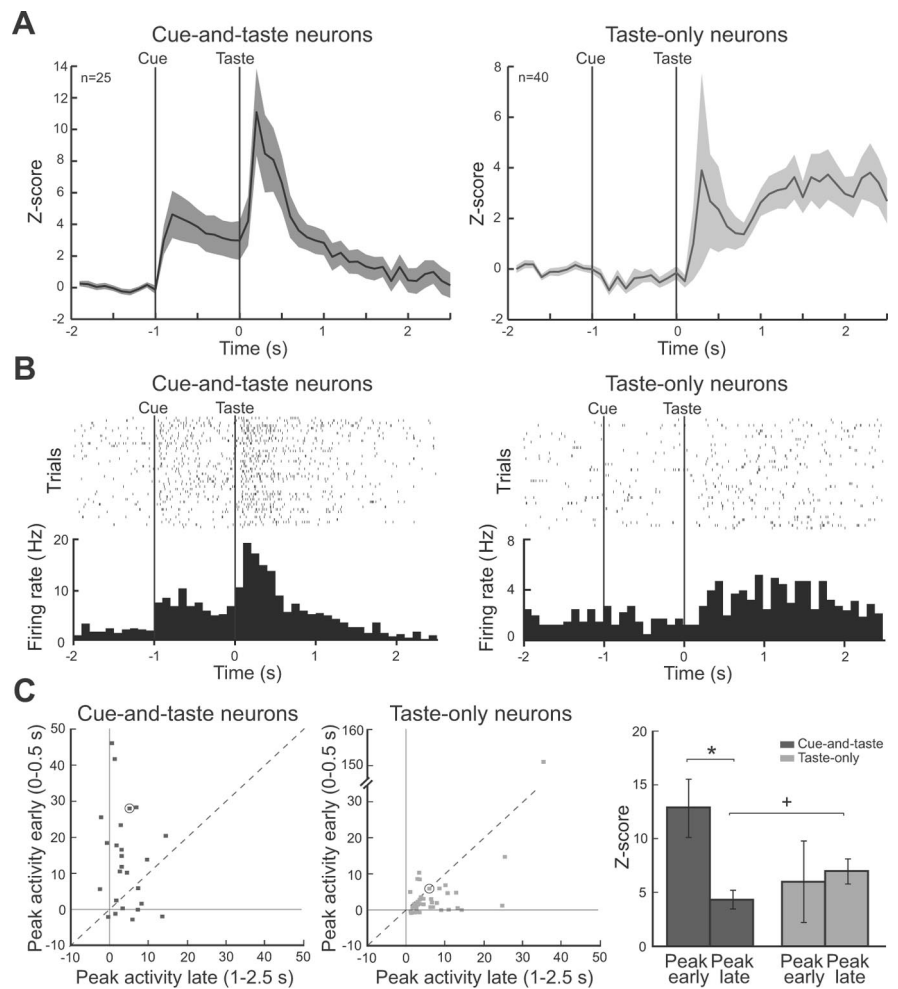

Figure 6. Behaviorally defined subpopulations of taste-selective GC neurons. *A*, Normalized population PSTHs for taste-selective GC neurons that responded to both cue and taste (*n* = 25, left) and taste-selective neurons that respond only to taste (*n* = 40, right). Taste delivery is at 0 s, cue onset is at −1 s. Shading around the trace: SEM. *B*, Raster plots and PSTHs for two representative neurons: a cue-and-taste neuron (left) and a taste-only neuron (right). Note the different time course of the responses. Cue onset is at −1 s and gustatory stimulation at 0 s. *C*, Quantification of early (0 −500 ms) and late (1000 −2500 ms) average peak firing activity for both cue-and-taste (dark gray squares; left) and taste-only (light gray squares; middle) neurons before thalamic inactivation. The cells selected as representative examples are marked with black circles. The histogram on the right details a comparison of across neurons average early and late *z*-scores for the two subpopulations. Cue-and-taste, dark gray; taste-only, light gray. *p < 0.01, +p < 0.05.

also differentially affected by thalamic inactivation; a larger reduction was observed for taste-only neurons, 66.6% of which lost taste specificity after MUS. In cue-and-taste neurons, in contrast, taste processing was less affected by VPMpc silencing. Whether the sparing of taste-selectivity was contingent upon conditioning or reflected a prelearning property of these neurons is at the moment unknown and will require further investigations.

Together our results highlight the importance of thalamic and extrathalamic sources of information in driving sensory and anticipatory responses in GC and further emphasize the task dependency of GC activity.

VPMpc and GC processing of gustatory information

The parvicellular portion of the VPMpc is considered to be the main source of gustatory information to GC. The VPMpc receives ascending inputs from the parabrachial nucleus (Karimnamazi and Travers, 1998), a fundamental brainstem relay in the rodent's taste pathway, and projects to the gustatory portion of the insular cortex (Allen et al., 1991; Maffei et al., 2012). Recordings from the
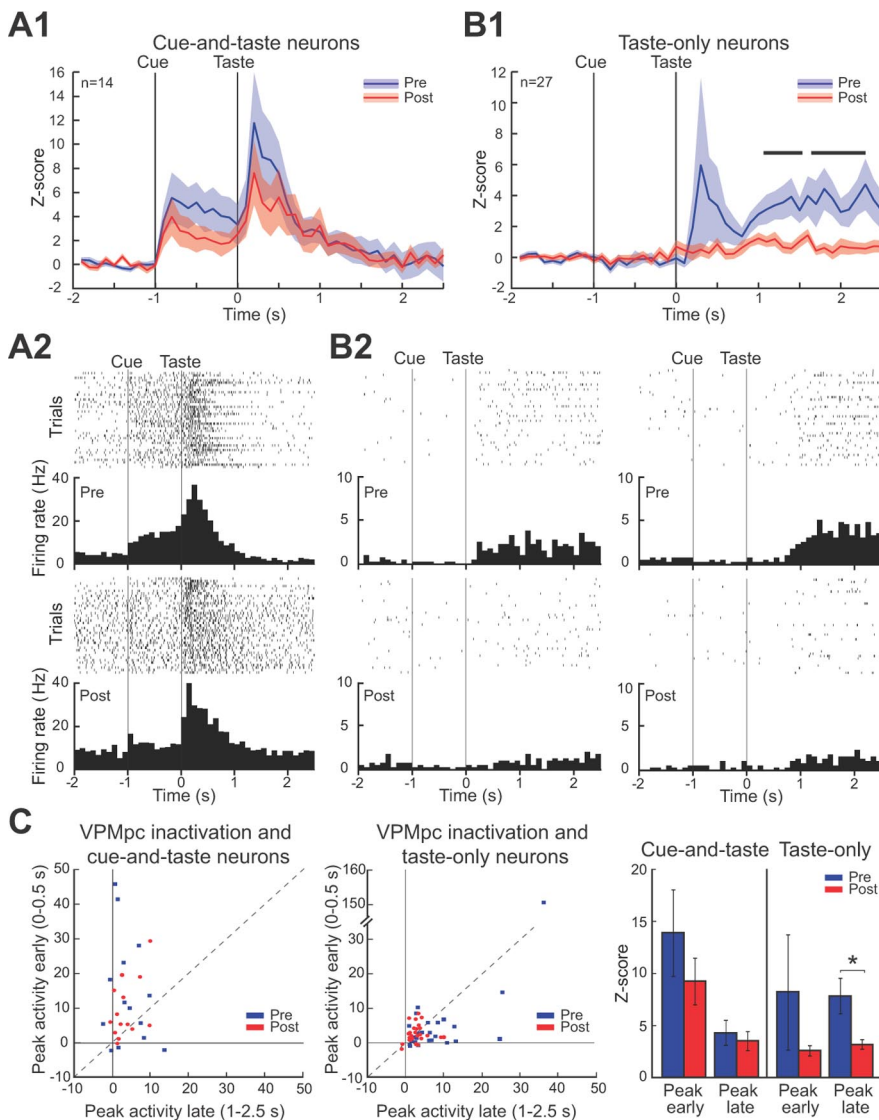
Figure 7. Differential sensitivity to VPMpc inactivation of cue-and-taste and taste-only neurons. **A1**, Population PSTHs for cue-and-taste neurons before (blue traces) and after (red traces) MUS inactivation. Cue-and-taste neurons showed no significantly different z-scores in response to cued tastants before and after VPMpc inactivation. The shading indicates the SEM. Cue onset is at −1 s and gustatory stimulation at 0 s. **A2**, Representative raster plot and PSTH for a cue-and-taste neuron before (top) and after (bottom) thalamic inactivation. **B1**, The population PSTH of taste-only neurons exhibits a significant inhibition after MUS inactivation. The solid horizontal line indicates the time points (∼1–2.5 s) for which the difference of activity is significant (p < 0.01). **B2**, Two representative raster plots and PSTHs for taste-only neurons before and after thalamic inactivation (left, neuron with short latency tonic response; right, neuron with long latency tonic response). Note how VPMpc inactivation greatly reduces responses of taste-only neurons. **C**, Quantification of early and late average peak firing activity for both cue-and-taste (left) and taste-only (middle) neurons before (blue squares) and after (red circles) thalamic inactivation. The histogram on the right details the across neuron average peak activity for cue-and-taste (left side) and taste-only neurons (right side) before (blue bars) and after (red bars) inactivation. No significant change in response after thalamic inactivation was observed for the cue-and-taste neurons. The taste-only subpopulation exhibited significantly reduced (p < 0.01) late activity by infusion of MUS. Significance: *p < 0.01.

lamic inactivation in both the sets of experiments. The results from the experiments relying on cue-taste association further extended this observation by showing that the responses that are less affected by thalamic inactivation are those from neurons processing both taste and anticipatory cues. The pattern of firing to stimuli of these neurons is indeed phasic, reaching its peak before 500 ms. The sources of activity for this population of neurons could be multiple. Firing in taste-and-cue could be driven by brainstem, i.e., parabrachial nucleus, and/or by limbic areas known to converge onto GC (Allen et al., 1991; Maffei et al., 2012). Recent results pointing to the basolateral amygdala (BLA) as a source of cue-related information (Samuelsen et al., 2012) suggest that BLA might also play a central role in determining part of the early responsiveness of cue-and-taste neurons. The presence of short-latency taste responses in BLA further support this possibility (Fontanini et al., 2009). Of course, considering the complexity of the connectivity of GC, also other areas, such as the lateral hypothalamus, the mediodorsal thalamus, frontal cortices, and somatosensory regions, may be involved in driving activity in the absence of VPMpc.

Anticipatory activity in GC and thalamic inputs

Mounting behavioral and electrophysiological evidence points to GC as an area responsible for processing taste-related anticipation (Ifuku et al., 2006; Saddoris et al., 2009; Mizuhiki et al., 2012; Samuelsen et al., 2012). Recent results have demonstrated that, after learning a cue self-administration task, a subset of GC neurons begins to respond to anticipatory cues with fast changes in firing rates (Samuelsen et al., 2012). The results in this article confirm the existence of cue-evoked activity in GC and further extend the finding to show that they can be observed also for classically conditioned cues. Our results also show that cue-responsiveness is spared by thalamic inactivation. Indeed the number of cue-responsive neurons was not significantly

VPMpc of anesthetized rats show that neurons in this nucleus encode multiple dimensions of gustatory experience, including temperature, oral touch, and, more importantly, chemosensation (Ogawa and Nomura, 1988; Verhagen et al., 2003). Our results, showing that taste responses to either uncued or cued tastants are heavily impacted by thalamic inactivation, emphasize the fundamental role of the VPMpc in providing GC with chemosensory information.

The data presented here, however, also suggests that the VPMpc is not the only source of taste-evoked responses. Phasic taste-evoked increases in firing appeared relatively spared after tha-

reduced by VPMpc inactivation. Analysis of the population PSTHs evoked by cues further confirmed the survival of cue responsiveness. We did observe a nonsignificant trend toward a slight reduction in cue responsiveness; however, it was present also in the case of saline infusions and it is likely attributable to time-dependent changes of internal state (Fontanini and Katz, 2005; de Araujo et al., 2006) or to a change in the general state of GC resulting from thalamic inactivation (Fontanini and Katz, 2006, 2008). Regardless of this trend, however, it is clear that information pertaining to anticipatory cues can still reach GC after the blockade of the VPMpc, a finding entirely consistent

with data showing that cue responses strongly depend upon amygdalar inputs (Samuelsen et al., 2012).

Interestingly, experiments based on permanent electrolytic lesions of the VPMpc propose its involvement in learning preparatory behaviors based on anticipatory cues (Reilly, 1998; Schroy et al., 2005; but see also Liang et al., 2012). Our results, which rely on temporary inactivation once the animal has learned the task, extend these findings. By showing that VPMpc is not necessary for mediating GC responses to learned anticipatory cues, our data suggest that the thalamic contribution to anticipation may be limited only to the initial learning of cue-taste associations.

Response dynamics in GC and subpopulations of neurons

GC neurons respond to tastants delivered via IOC with time-varying modulations of their firing rates (Katz et al., 2001; Jones et al., 2007; Grossman et al., 2008; Sadacca et al., 2012). Responses can occur at different latencies following the onset of the stimulus. Elevations or reductions of firing rates have been observed shortly after stimulus presentation (i.e., in the first ~250 ms), at an intermediate latency (~250–1000 ms after taste) and at a longer latency (~1000 ms after taste). While much work has been devoted to describing the informational content of responses in different epochs following taste presentation, an association between a specific pattern of firing and a specific neuronal population has yet to be established in GC.

By relying on a cue-taste association paradigm, we were able to identify in GC two neuronal populations on the basis of their responsiveness to taste and anticipatory cues and their susceptibility to VPMpc inactivation. At a population level these two groups of neurons showed different response dynamics. Average early firing activity in cue-and-taste neurons was significantly larger than that observed late, a result consistent with the idea that this group may contain more phasic responding neurons. In contrast, in taste-only neurons average late firing activity was at least as big as early activity, suggesting a more tonic pattern of response. The dissociation of the effects of thalamic inactivation further confirmed the diversity of these two populations. Responses of cue-and-taste neurons survived VPMpc inactivation, whereas responses in taste-only neurons were almost completely abolished by intrathalamic infusion of MUS.

To our knowledge our data are the first demonstration of an association between responsiveness to behavioral events, temporal dynamics, and thalamic connectivity in GC. Of course one should not conceptualize this association as the result of rigidly hard-wired labeled lines. First, it is important to notice that we did observe some within-group variability in response dynamics. Second, the fact that some neurons are driven primarily by thalamic or limbic sources does not exclude that according to the behavioral context they might also be modulated in subtle, but important ways by other inputs. Indeed, our data, showing that tonically firing, taste-only neurons are driven mostly by the thalamus, are not incompatible with the presence of other inputs that might exert modulatory influences on the late portion of a response. Recent results describing the effects of pharmacological inactivation of BLA on processing of uncued gustatory stimuli provides evidence in favor of this view (Piette et al., 2012).

Together, the results presented in this manuscript uncover unknown features of the thalamocortical organization of GC and emphasize the importance of studying taste processing against the background of different behavioral contexts.

References

Accolla R, Carleton A (2008) Internal body state influences topographical plasticity of sensory representations in the rat gustatory cortex. Proc Natl Acad Sci U S A 105:4010–4015. CrossRef Medline

Allen GV, Saper CB, Hurley KM, Cechetto DF (1991) Organization of visceral and limbic connections in the insular cortex of the rat. J Comp Neurol 311:1–16. CrossRef Medline

Ambroggi F, Ishikawa A, Fields HL, Nicola SM (2008) Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. Neuron 59:648–661. CrossRef Medline

Carleton A, Accolla R, Simon SA (2010) Coding in the mammalian gustatory system. Trends Neurosci 33:326–334. CrossRef Medline

de Araujo IE, Gutierrez R, Oliveira-Maia AJ, Pereira A Jr, Nicolelis MA, Simon SA (2006) Neural ensemble coding of satiety states. Neuron 51:483–494. CrossRef Medline

Erlich JC, Bialek M, Brody CD (2011) A cortical substrate for memory-guided orienting in the rat. Neuron 72:330–343. CrossRef Medline

Fontanini A, Katz DB (2005) 7 to 12 Hz activity in rat gustatory cortex reflects disengagement from a fluid self-administration task. J Neurophysiol 93:2832–2840. CrossRef Medline

Fontanini A, Katz DB (2006) State-dependent modulation of time-varying gustatory responses. J Neurophysiol 96:3183–3193. CrossRef Medline

Fontanini A, Katz DB (2008) Behavioral states, network states, and sensory response variability. J Neurophysiol 100:1160–1168. CrossRef Medline

Fontanini A, Grossman SE, Figueroa JA, Katz DB (2009) Distinct subtypes of basolateral amygdala taste neurons reflect palatability and reward. J Neurosci 29:2486–2495. CrossRef Medline

Ghazizadeh A, Ambroggi F, Odean N, Fields HL (2012) Prefrontal cortex mediates extinction of responding by two distinct neural mechanisms in accumbens shell. J Neurosci 32:726–737. CrossRef Medline

Grossman SE, Fontanini A, Wieskopf JS, Katz DB (2008) Learning-related plasticity of temporal coding in simultaneously recorded amygdala-cortical ensembles. J Neurosci 28:2864–2873. CrossRef Medline

Gutierrez R, Simon SA, Nicolelis MA (2010) Licking-induced synchrony in the taste-reward circuit improves cue discrimination during learning. J Neurosci 30:287–303. CrossRef Medline

Herry C, Ciocchi S, Senn V, Demmou L, Müller C, Lüthi A (2008) Switching on and off fear by distinct neuronal circuits. Nature 454:600–606. CrossRef Medline

Huguenard JR, McCormick DA (2007) Thalamic synchrony and dynamic regulation of global forebrain oscillations. Trends Neurosci 30:350–356. CrossRef Medline

Ifuku H, Nakamura T, Hirata S, Ogawa H (2006) Neuronal activities in the reward phase in primary and higher-order gustatory cortices of monkeys. Neurosci Res 55:54–64. CrossRef Medline

Jones LM, Fontanini A, Sadacca BF, Miller P, Katz DB (2007) Natural stimuli evoke dynamic sequences of states in sensory cortical ensembles. Proc Natl Acad Sci U S A 104:18772–18777. CrossRef Medline

Karimnamazi H, Travers JB (1998) Differential projections from gustatory responsive regions of the parabrachial nucleus to the medulla and forebrain. Brain Res 813:283–302. CrossRef Medline

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

Katz DB, Nicolelis MA, Simon SA (2002) Gustatory processing is dynamic and distributed. Curr Opin Neurobiol 12:448–454. CrossRef Medline

Kosar E, Grill HJ, Norgren R (1986) Gustatory cortex in the rat. II. Thalamocortical projections. Brain Res 379:342–352. CrossRef Medline

Liang NC, Norgren R, Grigson PS (2012) Pontine and thalamic influences on fluid rewards: Anticipatory contrast for sucrose and corn oil. Physiol Behav 105:595–606. CrossRef Medline

Maffei A, Haley M, Fontanini A (2012) Neural processing of gustatory information in insular circuits. Curr Opin Neurobiol 22:709–716. CrossRef Medline

Maren S, Hobin JA (2007) Hippocampal regulation of context-dependent neuronal activity in the lateral amygdala. Learn Mem 14:318–324. CrossRef Medline

Mizuhiki T, Richmond BJ, Shidara M (2012) Encoding of reward expectation by monkey anterior insular neurons. J Neurophysiol 107:2996–3007. CrossRef Medline

Ogawa H, Nomura T (1988) Receptive field properties of thalamo-cortical

taste relay neurons in the parvicellular part of the posteromedial ventral nucleus in rats. Exp Brain Res 73:364–370. Medline

Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates, Ed 6. Amsterdam: Academic/Elsevier.

Pfaffmann C, Norgren R, Grill HJ (1977) Sensory affect and motivation. Ann N Y Acad Sci 290:18–34. CrossRef Medline

Piette CE, Baez-Santiago MA, Reid EE, Katz DB, Moran A (2012) Inactivation of basolateral amygdala specifically eliminates palatability-related information in cortical sensory responses. J Neurosci 32:9981–9991. CrossRef Medline

Poulet JF, Fernandez LM, Crochet S, Petersen CC (2012) Thalamic control of cortical states. Nat Neurosci 15:370–372. CrossRef Medline

Pritchard TC, Norgren R (2004) Gustatory system. In: The human nervous system (Paxinos G, Mai J eds). New York: Academic.

Pritchard TC, Hamilton RB, Norgren R (1989) Neural coding of gustatory information in the thalamus of Macaca mulatta. J Neurophysiol 61:1–14. Medline

Reilly S (1998) The role of the gustatory thalamus in taste-guided behavior. Neurosci Biobehav Rev 22:883–901. CrossRef Medline

Sadacca BF, Rothwax JT, Katz DB (2012) Sodium concentration coding gives way to evaluative coding in cortex and amygdala. J Neurosci 32:9999–10011. CrossRef Medline

Saddoris MP, Holland PC, Gallagher M (2009) Associatively learned representations of taste outcomes activate taste-encoding neural ensembles in gustatory cortex. J Neurosci 29:15386–15396. CrossRef Medline

Samuelsen CL, Gardner MP, Fontanini A (2012) Effects of cue-triggered expectation on cortical processing of taste. Neuron 74:410–422. CrossRef Medline

Schroy PL, Wheeler RA, Davidson C, Scalera G, Twining RC, Grigson PS

(2005) Role of gustatory thalamus in anticipation and comparison of rewards over time in rats. Am J Physiol Regul Integr Comp Physiol 288: R966–R980. Medline

Smith DV, Travers JB (1979) A metric for the breadth of tuning of gustatory neurons. Chem Senses 4:215–229. CrossRef

Spector AC, Travers SP (2005) The representation of taste quality in the mammalian nervous system. Behav Cogn Neurosci Rev 4:143–191. CrossRef Medline

Stapleton JR, Lavine ML, Wolpert RL, Nicolelis MA, Simon SA (2006) Rapid taste responses in the gustatory cortex during licking. J Neurosci 26:4126–4138. CrossRef Medline

Steriade M (2000) Corticothalamic resonance, states of vigilance and mentation. Neuroscience 101:243–276. CrossRef Medline

Stone ME, Maffei A, Fontanini A (2011) Amygdala stimulation evokes time-varying synaptic responses in the gustatory cortex of anesthetized rats. Front Integr Neurosci 5:3. Medline

Verhagen JV, Scott TR (2004) Artificial neural network analysis of gustatory responses in the thalamic taste relay of the rat. Physiol Behav 80:499–513. CrossRef Medline

Verhagen JV, Giza BK, Scott TR (2003) Responses to taste stimulation in the ventroposteromedial nucleus of the thalamus in rats. J Neurophysiol 89: 265–275. Medline

Yamamoto T, Yuyama N, Kawamura Y (1981) Cortical neurons responding to tactile, thermal and taste stimulations of the rat's tongue. Brain Res 221:202–206. CrossRef Medline

Yamamoto T, Matsuo R, Kiyomitsu Y, Kitamura R (1989) Taste responses of cortical neurons in ad libitum ingesting rats. J Neurophysiol 61:1244–1258. Medline