

# Neural Signature of Taste Familiarity in the Gustatory Cortex of the Freely Behaving Rat

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**Bahar, Amir, Yadin Dudai, and Ehud Ahissar.** Neural signature of taste familiarity in the gustatory cortex of the freely behaving rat. *J Neurophysiol* 92: 3298–3308, 2004. First published June 22, 2004; doi:10.1152/jn.00198.2004. Ample data indicate that the gustatory cortex (GC) subserves the processing, encoding, and storage of taste information. To further elucidate the neural processes involved, we recorded multi-unit activity in the GC of the freely behaving rat as it became familiar with a novel tastant. Exposure to the tastant was performed over three 40- to 50-min sessions, 24 h apart. In each session, the tastant was presented repeatedly, 1 s at a time, with 10- to 12-s inter-trial intervals. The neural response to the tastant typically lasted 7 s. Our results show that the average neuronal response to the tastant increased as this tastant became familiar, but this increase was detected only during the last 5 s of the response. The increased response was not generalized to another tastant. Furthermore, our analysis suggests that specific neuronal populations subserves the processing of familiarity of specific tastants. The signature of familiarity was not detected in the course of the familiarization session, but only on the subsequent day, suggesting that its development involves slow post-acquisition processes. Our data are in line with the notion that GC neurons process multiple taste attributes, familiarity included, during different temporal phases of their response. The data also suggest that by default the brain considers a taste stimulus as novel, unless proven otherwise.

## INTRODUCTION

The ability to discriminate a novel taste stimulus from a familiar one carries important survival benefits, because the unfamiliar foodstuff may poison (Bronson 1967). Attempts have been made to identify and decode the neural mechanisms that subserves familiarity of sensory inputs (Berman et al. 2000; Brown and Bashir 2002; Holscher et al. 2003; Leveroni et al. 2000; Li et al. 1993; Tulving et al. 1996; Vankov et al. 1995). The study of familiarity raises several experimental challenges. Familiarity cannot be gauged on an absolute scale, and it depends on the sensory history of the subject. Taste provides a convenient sensory stimulus for the study of familiarity. Tastants are conveniently controllable, and incidental exposure to a novel tastant is a salient experience, which induces the formation of a long-term memory of that tastant (Berman et al. 1998; Bures et al. 1998).

The rat gustatory cortex (GC) has been implicated in the acquisition, consolidation, and retention of taste familiarity and taste associations (Kiefer and Braun 1977; Rosenblum et al. 1993; Yamamoto et al. 1994). Spiking activity of GC neurons reflects both chemosensory and somatosensory information (Hanamori et al. 1998; Yamamoto et al. 1989); these two components can be

partially separated along the temporal dimension of the neural response (Katz et al. 2001). It has further been reported that exposure to a novel tastant, as opposed to a familiar one, activates multiple neurotransmitter and intracellular signal transduction systems in the GC and that this activation is obligatory for the encoding and retention of long-term taste memory (Berman et al. 2000; Ferreira et al. 2002; Guiterrez et al. 1999; Rosenblum et al. 1997). Activation of the aforementioned systems outlasts taste presentation, develops over a time scale of minutes, and persists for hours. It is not yet known which neuronal events trigger these long-lasting processes and how the encoded information is related to the familiarity of the tastant processed during active stimulus sampling. It is plausible to assume that the spiking activity of GC neurons, known to process taste-related information within a fraction of a second (Halpern 1985), subserves familiarity processing at the time of stimulation and contributes to the immediate behavioral response to the taste.

Here, we examined how spiking responses of GC multi-units evolved as rats became familiar with a novel taste stimulus. Tastants were available for only 1 s during each trial to potentially allow for better separation of somatosensory and chemosensory inputs. Our results show that GC units signal taste familiarity at a delayed temporal phase of the response. Our analysis suggests that specific neuronal populations participate in the processing of familiarity for specific tastants. Furthermore, the neural signature of familiarity was correlated with familiarization with a specific tastant rather than with any tastant. This signature was not evident during the first session of exposure to the novel tastant (lasting ~45 min), but rather only in a second session, 24 h later. Thus persistent cortical representation of taste familiarity requires slow post-acquisition processing to develop.

## METHODS

### *Animals*

Male Wistar rats (9–10 wk old, 250–300 g) were caged individually at 22 ± 2°C in a 12-h light/dark cycle, with food ad libitum. Ten rats participated in recording of neural activity; 24 rats participated in behavioral assessment of taste familiarity. All experiments were approved by the Animal Care and Use Committee of the Weizmann Institute of Science.

### *Behavioral assessment of taste familiarity*

We determined behavioral familiarization to a tastant by using a protocol of latent inhibition (Lubow 1989) of conditioned taste aver-

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sion (CTA). This protocol quantifies familiarity in terms of the decremental effect of pre-exposure on the ability of a taste to enter into association in subsequent CTA training to the same taste. Rats that were not subjected to recording sessions ( $n = 6$  in each group) were subjected once, or twice, to a session of tastant drinking (sucrose, 5% wt/vol), under the same experimental setup and conditions used during recordings (see below). Two additional control groups were subjected to the same protocols, but water was used instead of a tastant. Twenty-four hours after the last session, all the rats were trained in a CTA protocol as described in Bahar et al. (2003). Briefly, they were presented with the tastant, and 40 min later, they were injected intraperitoneally with the malaise-inducing agent, LiCl (0.15 M, 2% body weight). CTA learning was quantified 48 h later in a 10-min multiple-choice test, in which the rats were allowed free access to an array of six pipettes: three with 5 ml sucrose each and three with 5 ml water each. Fluid consumption during the test was measured, and an aversion index was calculated. Aversion index was defined as  $\{[\text{ml of water}/(\text{ml of water} + \text{ml of sucrose})] \times 100\}$  consumed during the test. Hence, an aversion index  $>50$  implies preference of water over taste.

### Recording settings

Training and recording sessions were conducted in a double-wall sound attenuating chamber (I.A.C.). The test cage (transparent Plexiglas;  $42 \times 25 \times 30$  cm) was fully covered with black cloth apart from one wall to allow a video camera, situated outside the cage, to monitor the rat's activity. A small amount of wood shavings from the home cage was placed in the training and recording cage to increase contextual familiarity. An optical lickometer (E24-01, Coulbourn Institute) and a multi-barrel pipette that allowed the delivery of multiple tastants were placed behind a  $4.5 \times 4.5$  cm<sup>2</sup> opening in the wall. Each barrel was connected to 0.2-mm-ID plastic tubing, connected to a 10-ml pipette via an electric pin valve (Angar Scientific). The opening in the cage wall was hidden behind a plastic gate driven by a small servoengine (HS-300, Hitech). A PC controlled and monitored the inputs from the optical lickometer and the openings of the valves and gate at a rate of 1 kHz. Another PC recorded neural activity. The two computers were synchronized via parallel ports.

### Behavioral procedures

Rats were trained on the cued drinking task, described below, 1 wk before and 1 wk after implantation of the electrodes. At the beginning of training, rats were water deprived in their home cages for 24 h. In the subsequent 5 days, they were transferred to the test cage for 45–60 min daily. In the test cage, they were handled by the experimenter (e.g., a light touch by the experimenter hand, ~2 min) and trained to perform the drinking task with water as the stimulus (~150 trials daily). Each trial started with an auditory cue that signaled the opening of the gate (800 Hz, 100 ms); the rat was then given an opportunity to lick the pipette. The optical lickometer monitored initial contact with the pipette, as well as licking patterns. On initiation of drinking, three aliquots of liquid were delivered (total volume,  $50 \pm 5$   $\mu$ l) over a period of 900 ms. At the end of this period, another auditory cue (3,000 Hz, 100 ms) signaled the rat to remove its head from the pipette. Immediately following the cue, the gate was partly closed for 200 ms, preventing the rat from continuation of drinking but allowing it to remove its head, and then it was closed completely. The next drinking trial followed 10–12 s later (randomly). It took the rat 2–3 training days to learn the task, at which time it showed clear signs of anticipation following the first auditory cue (e.g., moving in the direction of the gate) and removed its head from the lickometer on the second auditory cue. Following 5 days of training, the rat had free access to water for 3–4 days, after which it was implanted with chronic electrodes (see *Implantation of electrodes*). Following recuperation from surgery (7–14 days), the rat was water deprived for 24 h

and trained again for 4 days as described above. On the fifth day, it performed 150 drinking trials while the head-stage was attached to the electrode to familiarize it with the recording apparatus.

### Implantation of electrodes

Rats were deeply anesthetized with sodium pentobarbital (85 mg/kg, ip). Additional doses of pentobarbital were applied during surgery if needed. Rats were restrained in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with blunt ear bars. The skull was exposed, and a small opening was made just above the right insular cortex (IC; anteroposterior, +1.2 mm, lateral, +4.8–5.2 mm, relative to bregma; Paxinos and Watson 1998). An additional four openings were made at different locations on the skull to allow insertion of screws that secured the dental cement and served as ground. Multi-wire electrodes were made of eight-stainless steel Teflon coated microwires (50  $\mu$ m diam; impedance, 200–500 kOhms, at 1 kHz), arranged in a bundle (~150–250  $\mu$ m diam; NB-labs, Dennison, TX; Nicolelis et al. 1997). The electrode was placed just above the IC, and the ground wire was attached to the ground screws. The electrode was lowered slowly at a rate of 100–200  $\mu$ m/1–5 min, until the IC was reached (4.7–5 mm below dura). In two rats, an additional two wires (120  $\mu$ m each) were implanted in the right masseter muscle to record EMG activity during the experiments. After final placement of the electrode, acrylic dental cement was applied around the plastic connector to secure the electrode to the skull. Rats were injected with 0.2 ml of Pen-Strap to prevent infection. They were allowed 7–14 days of recuperation with food and water available ad libitum.

### Recording sessions

Recording experiments commenced immediately after the last training session. Each experiment lasted 1 wk, with an inter-experiment period of 1 wk. Each experiment was dedicated to one tastant. NaCl (0.155 M) and sucrose (0.146 M) were used as tastants. Water was double-distilled, either when used to dilute the tastants or as a stand-alone stimulus. NaCl and sucrose were selected because they do not evoke the motor activity typical to neophobic reactions (Grill and Norgren 1978; Bahar and Dudai, unpublished data). Six rats were subjected to two recording experiments, one for each tastant stated above. Four additional rats were subjected to only one recording experiment (due to technical considerations). Each experiment included three tastant sessions, designated NOVEL, FAMILIAR1, and FAMILIAR2, which corresponded to the first, second, and third exposure to the same tastant, respectively. These sessions were performed 24 h apart. Each tastant session included four alternating blocks of water (W) or tastant (T) application (designated W1, T1, W2, T2, and given in that order). Each block consisted of 35 drinking trials, so that overall, each session consisted of 70 trials to water and 70 trials to tastant (Fig. 1A). Each session lasted 40–50 min. Two squirts of water (200  $\mu$ l each) flushed the drinking pipette at the end of each block. Recordings from two additional sessions of water only were performed before and after the aforementioned recording sessions, bringing the total number of recording sessions in each experiment to seven. Each water session was divided into four blocks (W1–4; see Fig. 1A) that were later analyzed as the blocks in tastant sessions (see *Data acquisition and analysis*). Following each session, rats were given 30 min to consume their daily water ration; between experiments, they were given free access to food. Since rats manage to consume only  $<50\%$  of their daily water ration (7/~15 ml) during each recording session, we did not expect a major decline in the level of thirst during recordings; this was also evident behaviorally because rats showed high motivation to drink at the home cage following each session.

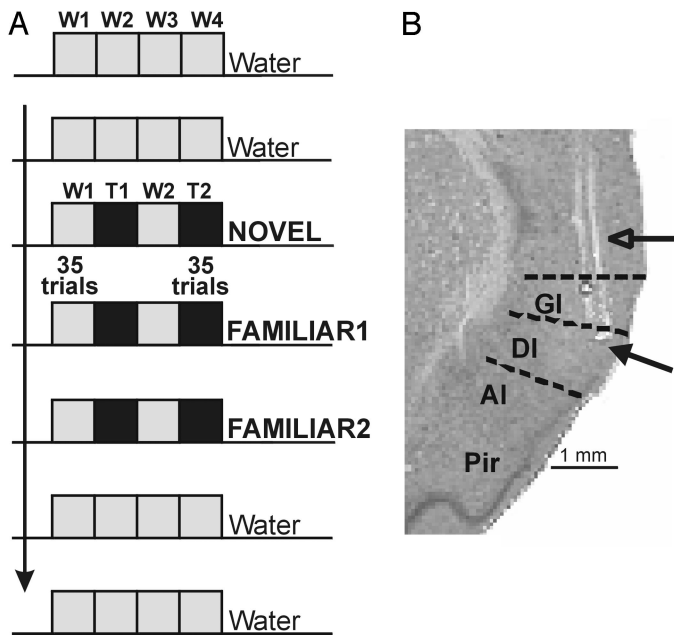


FIG. 1. *A*: schematic diagram of the experimental protocol. Each experiment to a specific taste (NaCl or sucrose) started and ended with 2 sessions of water drinking (Water), divided into 4 blocks of 35 drinking trials each (W1–4). In NOVEL, FAMILIAR1, and FAMILIAR2 sessions, rats drank water and tastant in alternating blocks of 35 trials each (W1, W2, water, gray blocks; T1, T2 tastant, black blocks). The inter-session interval was 24 h; for details see METHODS. *B*: multi-wire electrode placement in the rat insular cortex. The coronal brain section (+1.2 mm anterior to bregma) depicts the coagulation lesions that mark electrode tips location (filled arrow), as well as the track of the wires (empty arrow). GI, granular insular cortex; Pir, piriform cortex; DI, dysgranular insular cortex; AI, agranular insular cortex. Adapted from Paxinos and Watson (1998).

#### Data acquisition and analysis

During recordings, the electrodes were connected to a voltage-follower head stage (NB-Labs). The analogue-recorded signals were amplified and filtered (8,000–15,000 $\times$  gain; 0.5- to 4.5-kHz band-pass filter; MCP plus, Alpha-Omega, Nazareth, Israel). The signals were digitized at a sampling rate of 30 kHz/channel (DAP 5200, Microstar Laboratories), displayed on a computer screen, and stored using Alpha-Map acquisition program (Alpha-Omega). To monitor facial motor activity, we recorded the EMG of the facial masseter muscle, one of the main muscles responsible for mouth movements during ingestion. EMG activity was sampled at a rate of 0.5 kHz (2- to 200-Hz band-pass filter) and quantified by calculating the number of times activity crossed  $2.5 \times$  SD of the inter-trial interval activity (this threshold captured activity that deviated from the background noise with a confidence of  $>98\%$ ). EMG values varied between rats, and to combine them, we normalized the data points to the highest value before averaging. We also monitored oro-facial activity using a video monitor. Data were collected from recording experiments that showed an increase in taste/water ratio during the LP in two rats. The following typical oro-facial behaviors (Grill and Norgren 1978) were counted following the end of each drinking trial: mouth movements lasting  $<2$  or  $>2$  s; tongue protrusions lasting  $<2$  or  $>2$  s; chin rubbing  $\leq 5$  s.

Spike waveforms were sorted off-line using ASORT program (Alpha-Omega) that employed principle component analysis (see Fig. 3 for examples of sorted units). Standard methods of analysis were used (Sosnik et al. 2001). Peristimulus time histograms (PSTHs) were constructed at 1-ms resolution and smoothed by a low-pass filtering cutoff frequency of 10 Hz. Most recorded units showed a lingering response (above spontaneous firing rate) to 1 s of drinking stimulation, which lasted  $\leq 7$  s after drinking onset. We divided the response into

three temporal phases: early phase (EP), 0–1 s after drinking onset; middle phase (MP), 1–2 s after drinking onset; and late phase (LP), 2–7 s after drinking onset (see PSTHs in Fig. 3). This division was based on the following rationale: EP was the period in which the stimulus was presented. In addition, the neural responses and EMG recordings of the facial muscle during this phase showed strong rhythmic activity at 5–10 Hz (Fig. 4), typical for licking activity (Katz et al. 2001); MP was the poststimulus period in which 5- to 10-Hz oscillations were still evident in the EMG recording and neural responses; in LP, oro-facial activity declined markedly, but neural activity was still above pretrial level (Fig. 4). All further analyses were performed according to these response phases.

Taste responding units (TRUs) were identified for each response phase by comparing the evoked responses (spike count over the entire phase) to taste and water. To adjust the spike counts into a normal distribution, a square-rooting transformation was applied. A neuron was considered a TRU in a given session and a given response phase if its response to taste differed from its response to water at a confidence of  $P < 0.05$  (2-tail *t*-test). The differences in percentages of TRUs were compared between the sessions and across all experiments using Wilcoxon signed-rank test. This test compares the binomial proportions for two samples and takes into account the magnitude of differences within pairs of scores.

To investigate responses across the whole population, an average spike count for each phase and for each trial was calculated based on unit-by-unit spike counts. Data from water blocks (W1, W2) or taste blocks (T1, T2) were combined, respectively. To analyze “water sessions,” spike counts were calculated for each phase as above. In this analysis, blocks W2 and W4 during the “water sessions” were considered analogous to taste blocks during taste sessions (T1, T2), and therefore their data were combined. Blocks W1, W3 during the “water sessions” were considered analogous to water blocks in the taste sessions, and their data were also combined (see Fig. 1A).

Graphs that describe the distribution of taste/water ratios were constructed by dividing the average response to taste by that of water on a unit-by-unit base. The resulting distributions were usually not normal and therefore compared by Mann-Whitney *U* test. Exponential smoothing of the distributions was used for visualization (damping factor = 0.3).

PSTHs representing population responses (e.g., Fig. 8A) were constructed from the averaged activity of multiple multi-units and smoothed by a convolution with a low-pass zero-phase Butterworth filter with cutoff frequency at 2 Hz. This low smoothing time constant was chosen to emphasize the effects at the slow time scales, typical for the taste system (Halpern 1985). Data points in the graphs that describe response dynamics along the sessions were constructed by averaging six trials for each. After testing several bin sizes, we selected this bin size as the best in representing the data.

To calculate the correlation between the familiarity response to a specific tastant and the tendency of the recorded population to respond specifically to that tastant, we correlated an index of familiarity response with an index of response to a tastant in the EP and MP during the NOVEL and FAMILIAR1 sessions. The index of familiarity response was defined as [(spike count during the LP in the FAMILIAR1 session – spike count during the LP in the NOVEL session)/(spike count during the LP in the FAMILIAR1 session  $\pm$  spike count during the LP in the NOVEL session)]. The index of response to a tastant was defined as [(mean spike count to a specific tastant during the EP and MP in the NOVEL and FAMILIAR1 sessions – mean spike count to water during the EP and MP in the NOVEL and FAMILIAR1 sessions)/(mean spike count to a specific tastant during the EP and MP in the NOVEL and FAMILIAR1 sessions + mean spike count to water during the EP and MP in the NOVEL and FAMILIAR1 sessions)]. Correlations here and in the EMG analysis were described by the Pearson product-moment correlation coefficients. Significance level was determined by Bonferroni test of probability.

## Histology

Following the last recording session, rats were deeply anesthetized with sodium pentobarbital. A DC current was passed through each wire (35  $\mu$ A, 7 s) to induce a lesion. Next, rats were perfused with saline and then with 5% formalin. The brains were removed and fixed overnight with 5% formalin and 30% sucrose. Brain sections (40  $\mu$ m) were labeled with Nissel-stain and examined to locate electrode tips position (Haidarliu et al. 1999).

## RESULTS

### Histology

Figure 1B shows a representative lesion made by the tips of a multi-wire bundle electrode (8 wires), located in the dysgranular zone of the insular cortex (DI). Other recordings were also made in this area. This area is known to contain cells that respond to taste stimulation (Katz et al. 2001; Yamamoto et al. 1989), and its functional ablation impairs taste learning (Berman et al. 2000). Bundle wire tips were usually spread across several cortical layers.

### Behavior

To quantify behavioral familiarization with a taste stimulus under our experimental protocol (see Fig. 1A), we used a protocol of latent inhibition (Lubow 1989) of CTA. In this protocol, familiarization with a taste stimulus reduces its associability in subsequent CTA training; thus the degree of reduction in CTA performance can serve as a measure of the relative familiarity by which the taste stimulus was perceived during training (Rosenblum et al. 1997). Rats that were pre-exposed to sucrose under the same conditions used in recordings (but not subjected to recording themselves), showed a significant decrease in CTA performance [aversion index,  $71 \pm 8$  (SE) and  $62 \pm 4$  for 1 and 2 pre-exposures, respectively) compared with rats that were pre-exposed to water alone ( $94 \pm 2$ ; ANOVA,  $F_{(2,15)} = 12$ ,  $P < 0.001$ ; the difference between the aversion indices of groups preexposed once or twice to sucrose was not significant,  $P = 0.4$ , Tukey test; Fig. 2).

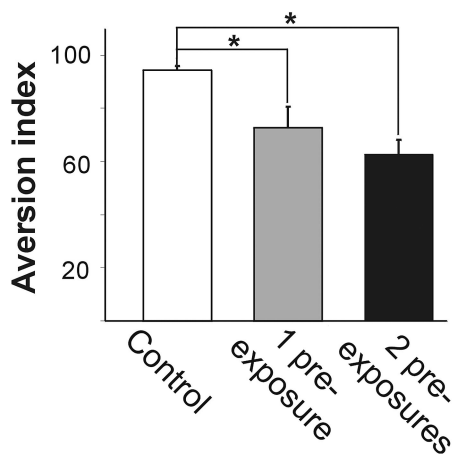


FIG. 2. Decreased conditioned taste aversion (CTA) following familiarization to a taste stimulus. Groups: 1, pre-exposure, rats pre-exposed once to sucrose before CTA training; 2, pre-exposures, pre-exposed twice to sucrose before CTA training; Control, rats without prior presentation to sucrose before CTA training. Values are means  $\pm$  SE,  $n = 6$  each; \*Statistical significance (here and following figures).

### Neural data

The neural data were obtained from 10 rats. Six rats participated in 2 experiments each, and four rats participated in only 1 experiment; hence data were used from 16 experiments altogether. Following off-line sorting, most signals were identified as belonging to multi-units, which will be referred to herewith as units (based on spike shapes and inter-spike interval analysis; only 4% were clear single units; see Fig. 3). No attempt was made therefore to trace units from 1 day to another, and analysis was at the population level throughout. For the NOVEL and FAMILIAR1 sessions, 169 units were recorded for each session. In 10 of 16 experiments (129 units), FAMILIAR2 sessions were also performed. Spontaneous firing rates were generally low, with an average of 0.92 spikes/s and a median of 0.74 spikes/s; rates ranged from 0.01 to 4.8 spikes/s. Mean spontaneous firing rate did not differ significantly between NOVEL, FAMILIAR1, and FAMILIAR2 sessions (ANOVA,  $F_{(2, 464)} = 1.1$ ,  $P > 0.2$ ).

### Analysis of taste responding units

We calculated the percentage in the total population of units whose evoked responses were significantly different between tastant and water (whether responses were higher or lower to a tastant compared with water). These units were dubbed TRUs. Basing our calculations on the response during the LP yielded the highest percentage of TRUs under all conditions. Percentages of TRUs in the LP were 27% (45/169 units), 36% (60/169 units), and 34% (44/129 units) for NOVEL, FAMILIAR1, and FAMILIAR2 sessions, respectively (Fig. 5). However, Wilcoxon signed-ranks test showed no significant statistical differences in the percentages of TRUs between sessions during any of the phases (e.g., LP: NOVEL-FAMILIAR1:  $P = 0.27$ ; NOVEL-FAMILIAR2,  $P = 0.95$ ; FAMILIAR1-FAMILIAR2,  $P = 0.9$ ).

### Analysis across the entire population

We next calculated the spike count for each phase averaged across the total population of recorded units, regardless of their response to taste or water. Figure 6 shows that, while the average spike count was not significantly different between taste and water in the NOVEL session during any phase, there was a significant increase in average spike count for taste compared with water in the FAMILIAR1 and FAMILIAR2 sessions during the LP only [average response to water vs. tastant,  $6.9 \pm 0.3$  vs.  $7.1 \pm 0.3$ ,  $7 \pm 0.4$  vs.  $8.3 \pm 0.4$ ,  $6.6 \pm 0.2$  vs.  $7.7 \pm 0.3$  spikes/phase; paired  $t$ -test,  $P = 0.48$ ,  $P < 0.001$ , and  $P < 0.001$  in NOVEL ( $n = 169$ ), FAMILIAR1 ( $n = 169$ ), and FAMILIAR2 ( $n = 129$ ), respectively]. As a control, we performed a similar spike count analysis on neural responses recorded during “water sessions” only (conducted before and after the NOVEL and FAMILIAR2 sessions, respectively; see Fig. 1A). This analysis showed that the average activity of the entire population was highly similar in the different blocks, within and across sessions during all phases, when rats drank water only (Fig. 6, insets). These data suggest that the increased response to a tastant during the familiar sessions was not a result of unstable recordings or the order of tastant and water blocks within a session.

To further explore the dynamics of the familiarity response described above on a session by session basis, we calculated, for each unit, the ratio of its response to tastant versus water in each session. Figure 7 depicts ratio distributions among units

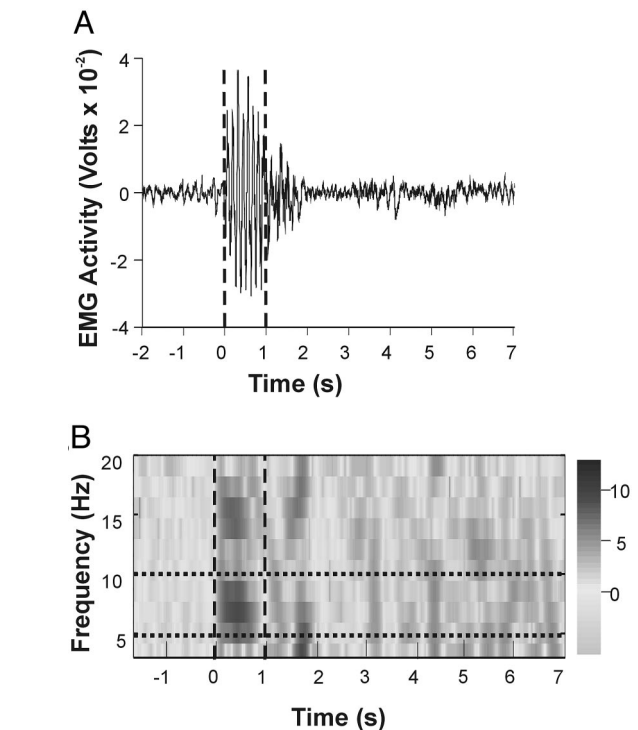
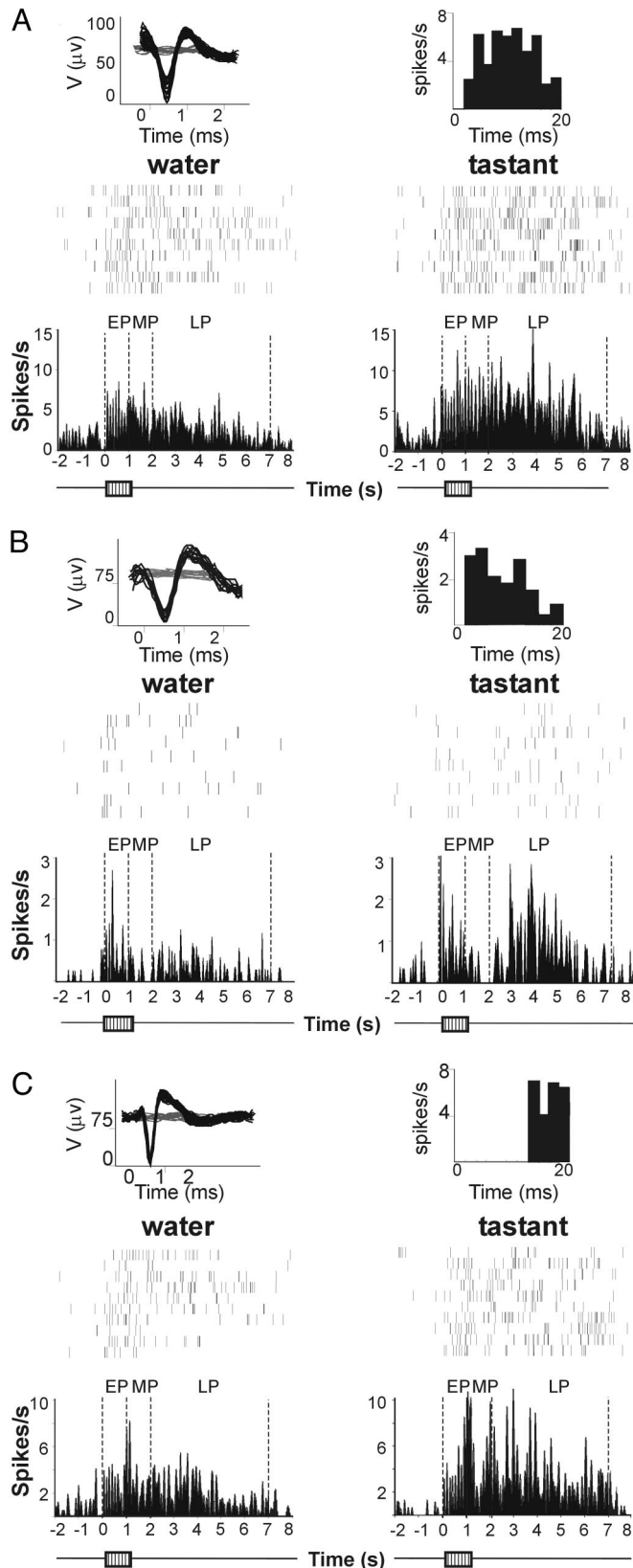


FIG. 4. Measures of oro-facial behavior during experimental trials. *A*: average EMG trace, averaged from 50 drinking trials recorded from the masseter muscle. EMG traces were triggered on the 1st touch made to the drinking pipette in each trial, as detected by the optical lickometer. *B*: spectrogram representing cortical multi-unit activity during task performance. Power (grayscale coded) was computed from the average response of the multi-unit during 50 trials of drinking a taste stimulus. Horizontal broken lines border the 5- to 10-Hz frequency. Frequencies of 5–10 Hz (typical for oro-facial movements; Katz et al. 2001) gained the highest power during the EP (0–1 s), to a lesser degree during the MP (1–2 s), and the least during the LP (2–7 s). In both panels, vertical broken lines border the 1 s of active licking.

recorded from two of the rats that participated in two experiments each (Fig. 7, *A* and *B*, for rat 1 and Fig 7, *C* and *D* for rat 2). In the population recorded from rat 1, the median of the distribution of sucrose versus water ratios increased significantly during the LP in FAMILIAR1 and FAMILIAR2 sessions compared with the NOVEL (Fig. 7*B*). Familiarization with NaCl did not change the median of the distribution significantly (Fig. 7*A*). In rat 2, a significant increase in NaCl versus water ratios in the population was observed on familiarization with this tastant (Fig. 7*C*) but not on familiarizing with sucrose (Fig. 7*D*).

Overall, the recorded population in 70% (7/10) of the rats showed significant increased taste/water ratio between NOVEL to FAMILIAR1 and FAMILIAR2 sessions during the LP only. The recorded units from rats that participated in two experiments, using different tastants, showed increased response to one tastant (5/6 rats), but never to both; this increase was

FIG. 3. Samples of recorded units in the gustatory cortex (GC). Stacked waveforms of representative multi-units (*A* and *B*) and a putative single unit (*C*) are drawn above raster plots and peristimulus time histograms (PSTHs) that show their responses to water (*left*) and tastant (sucrose; *right*). Rectangles under the PSTHs depict period of active drinking during each trial. Inter-spike interval histograms, plotted on a logarithmic scale (bin-widths increase by a factor of 1.5, starting with 1-ms bin), are shown next to the waveforms. PSTHs were divided into 3 phases (indicated by vertical broken lines): early (EP), middle (MP), and late (LP). Rasters depict a random sample of the trials that were used to construct the PSTHs.

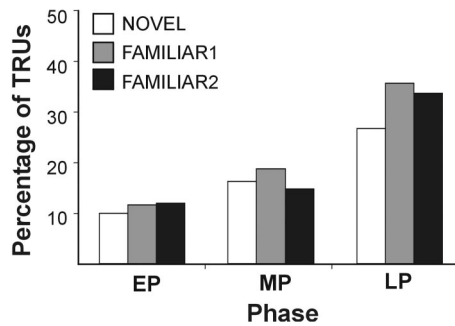


FIG. 5. Percentages of taste responding units (TRUs) in the total population. Although the percentage of TRUs, identified on the basis of differential response to tastant vs. water at the different phases, increased as the tastant became familiar, this increase did not reach statistical significance in any of the phases.

detected in 3/5 experiments to sucrose and 2/5 to NaCl, and was not confined to the first experiment in the series of two (2/5 experiments showed this increase when they were 1st in the series, the rest when they were 2nd). These data suggest that the increased response to familiar taste during the LP was not a general response to any familiar stimuli, nor was it related to rule learning underlying novel to familiar transformation. Rather, the data did correlate with the familiarization with a specific experienced tastant.

We also tested whether, for each rat, the recorded population that showed a familiarity response to a specific tastant during the LP tended to respond specifically to this tastant during the EP and MP, regardless of familiarity. No significant correlation was found between the familiarity response to a specific tastant and the tendency of each population to respond specifically to this tastant during the EP or MP (sucrose,  $r^2 = 0.06$ ,  $P = 0.5$ ; NaCl,  $r^2 = 0.09$ ,  $P = 0.4$ ).

#### Analysis of response dynamics

To explore the dynamics of the response to the familiar tastant, we averaged PSTHs taken from experiments in which the recorded population showed a significant increased response to familiar tastant (94 units). Figure 8A shows that the difference between the averaged response to familiar taste versus water was relatively stable during the entire LP (in FAMILIAR1 and FAMILIAR2 sessions; data of the latter not shown). To explore the dynamics of the response during the LP throughout the entire session, we averaged spike counts taken from groups of six trials within sessions (grouping by 5 or 7 trials yielded similar results). Figure 8B shows that the increased spike count to familiar taste during the LP was consistent along the entire FAMILIAR1 session and that no earlier increase of average activity could be observed during the NOVEL session. We also analyzed the response dynamics along the sessions for each unit individually. Along the NOVEL sessions, most units displayed a tendency to decrease their response to water and tastant stimulations [water, slope =  $-0.35 \pm 0.29$  (SD) spikes/block of 6 trials; taste, slope =  $-0.27 \pm 0.48$  spikes/block of 6 trials). During the FAMILIAR1 sessions, units also tended to decrease their response but to a lesser degree (water, slope =  $-0.07 \pm 0.22$  spikes/block of 6 trials; taste, slope =  $-0.17 \pm 0.42$  spikes/block of 6 trials). The average slope did not differ significantly between the response to taste and water in each session ( $P > 0.1$ ). We

also tested whether the balance between units that increased or decreased their tastant/water response ratio (“excited” and “inhibited,” respectively) remained constant during the NOVEL session. No change in the ratio of tastant/water response could be detected during the NOVEL session when units were divided into “excited” and “inhibited” by the tastant. The slopes of the tastant/water response ratios of the “excited” units were  $0.08 \pm 0.026$  and those of the “inhibited” units were  $0.05 \pm 0.024$ , with no significant difference between them ( $P = 0.254$ ).

#### Analysis of oro-facial activity

We monitored and analyzed several parameters related to oro-facial motor activity that could potentially affect the recorded neural responses. Figure 9A shows that rates of typical

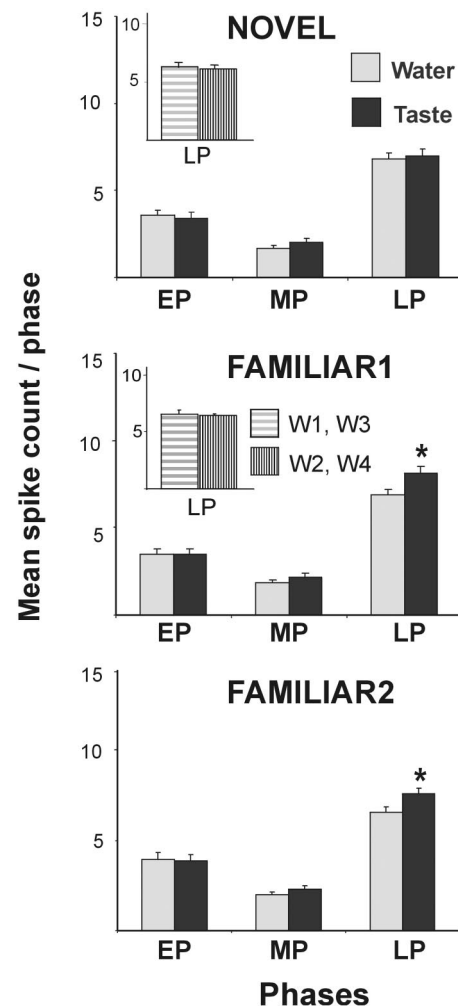


FIG. 6. Differential response to familiar taste revealed in the average spike count of the entire recorded population during LP. The average spike counts were significantly higher to taste vs. water in FAMILIAR1 and FAMILIAR2 sessions during the LP. The insets in NOVEL and FAMILIAR1 plots compare the average population responses during the LP between W1, W3 (gray horizontal stripes bars) and W2, W4 blocks (black vertical stripes bars), in the 2 water sessions that preceded the NOVEL sessions. The inset in the NOVEL plot depicts data taken from the 1st session, and the inset in FAMILIAR1 plot depicts the 2nd session. Average spike counts here and in the 2 water sessions conducted after the FAMILIAR2 session (data not shown) were highly similar in each phase. Error bars represent SE.

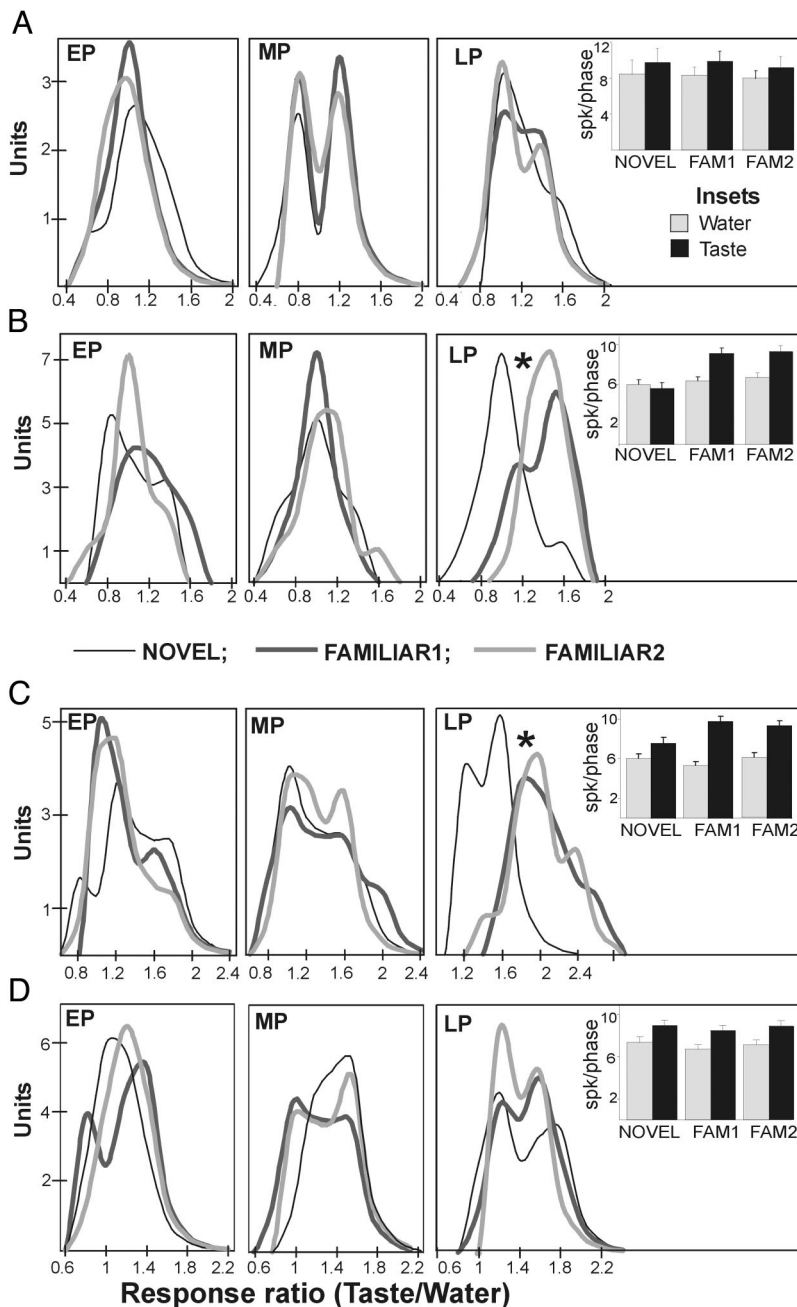


FIG. 7. Phase-wise distributions of response ratios (tastant/water) in the recorded population as a function of familiarity. The *x*-axis in each plot describes tastant/water ratio; *y*-axis describes the number of units found for a given ratio. *A* and *B*: data acquired from rat 1 exposed to NaCl (*A*,  $n = 8$  units for each session) and in a later experiment to sucrose (*B*,  $n = 15$  for each session). *C* and *D*: data acquired from rat 2 exposed to NaCl (*C*,  $n = 13$  for each session) and in a later experiment to sucrose (*D*,  $n = 15$  for each session). A significant shift to the right was observed in the distribution curves (i.e., units increased their tastant/water ratio) during LP between NOVEL and FAMILIAR1 sessions when rat 1 familiarized with sucrose (*B*; Mann-Whitney *U* test statistics = 15,  $P < 0.0001$ ) or when rat 2 familiarized with NaCl (*C*;  $U = 11$ ,  $P < 0.0001$ ). These shifts remained significantly different in FAMILIAR2 compared with NOVEL. *Insets*: actual average spike counts during the LP for each population. Responses to water and taste are depicted in gray and black bars, respectively. FAM1, and FAM2 are FAMILIAR1 and FAMILIAR2 sessions, respectively.

orofacial movements related to ingestion, monitored by a video camera during the experiments, were not significantly different between NOVEL and FAMILIAR1 sessions or between water and tastant blocks. We also found that the average number of licks per trial (during the EP) was similar among all sessions ( $7 \pm 0.3$  licks on average, data not shown). EMG recordings of the masseter muscle provided a more direct measure of orofacial activity. Figure 9*B* shows that EMGs recorded from two rats did not change significantly between exposures to water or tastant during the LP in either the NOVEL or FAMILIAR1 sessions. In contrast, the average spike count, recorded from the same rats and during the same experiments, increased significantly in response to the familiar tastant compared with water in the FAMILIAR session (Fig. 9*B*). To further explore the relevance of the facial muscular activity to the neural

response, we calculated the correlation between the EMG activity and the neural response on a trial-by-trial basis throughout the recording sessions and for each phase. The highest values of Pearson correlation coefficients were found during the EP ( $r^2 = 0.5$ ,  $P < 0.01$ ) compared with the MP and LP (Fig. 9*C*;  $r^2 = 0.18$  and  $0.13$  in MP and LP, respectively;  $P > 0.2$  for both).

#### DISCUSSION

The objective of this work was to contribute toward better understanding of the role of the GC in encoding, processing, and storing information about the familiarity of a taste item. To that end, we recorded the extracellular activity of multi-units in the GC of the freely behaving rat as it became familiar with a

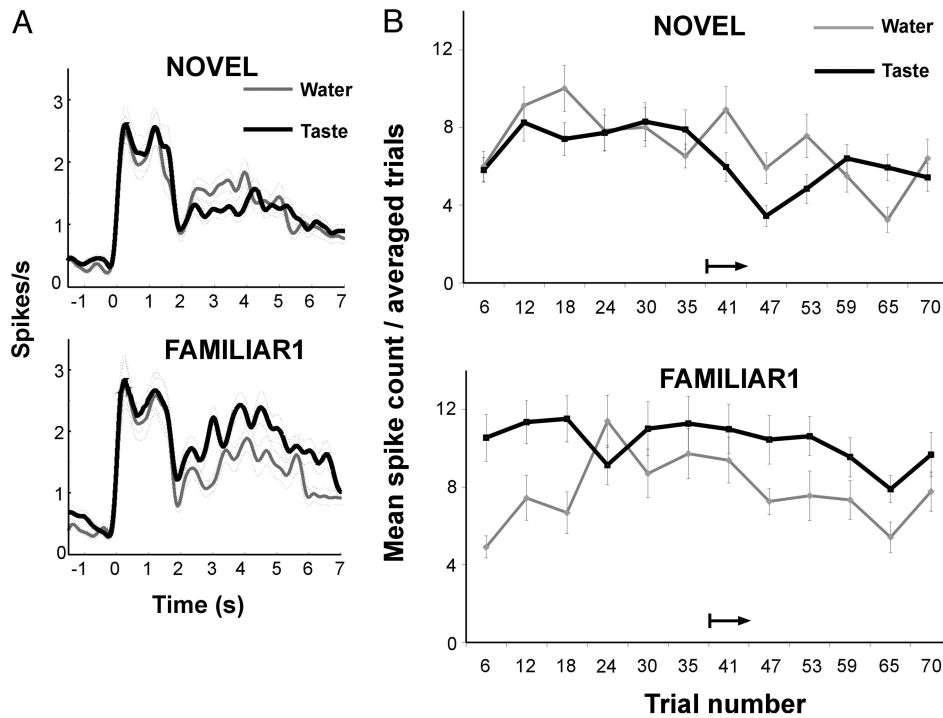


FIG. 8. Dynamics of the neural response to a familiar tastant. *A*: difference between the increased response to the familiar tastant vs. water was relatively stable throughout LP. PSTHs depict response shape averaged across the neural populations recorded in experiments showing a significant increased response to familiar tastants. Average PSTH of the FAMILIAR2 was highly similar to the PSTH of FAMILIAR1 and therefore not presented here. Thin gray lines depict  $\pm$ SE. *B*: sustained increase in response to tastant vs. water in the FAMILIAR1 session. Data were averaged for groups of 6 trials each (last data point in each block was averaged for 5 trials). Arrows denote initiation of the 2nd experimental block in the same session.

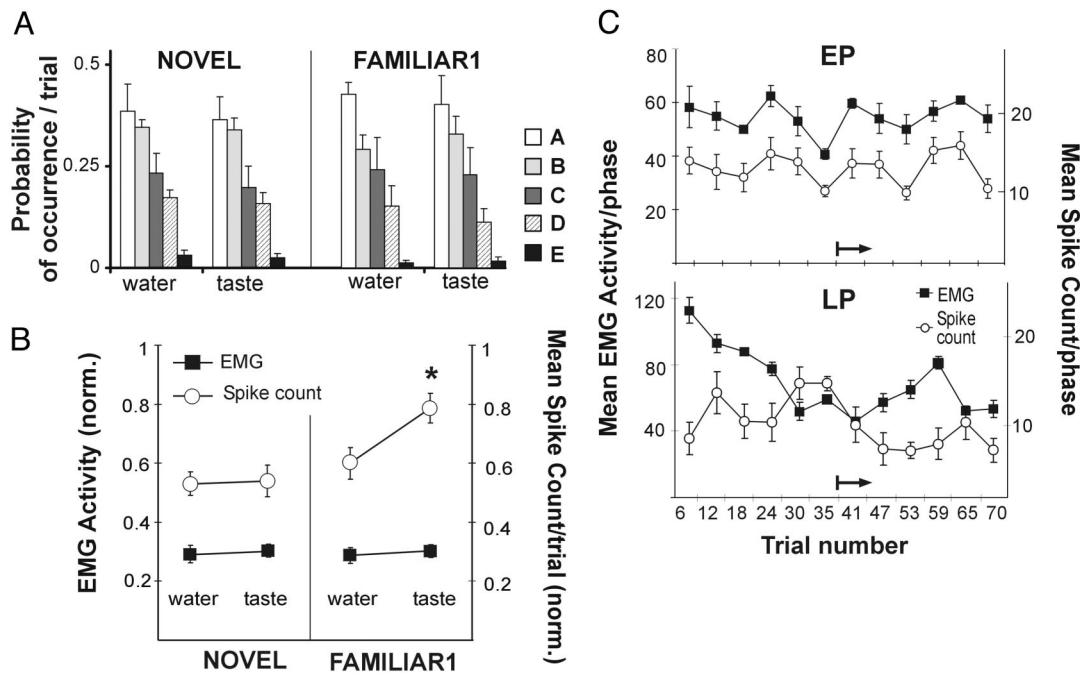


FIG. 9. Behavioral measures monitored during recordings. *A*: typical orofacial movements analyzed from video recordings of experiments that showed increased taste response and their probabilities of occurrence in a trial (mouth movements lasting  $<2$  and  $>2$  s in *A* and *B*, respectively; tongue protrusions lasting  $<2$  and  $>2$  s in *C* and *D*, respectively; chin rubbing, *E*; time is after drinking offset). ANOVA showed no main effects of sessions, or stimuli, nor any interaction between the 2 (*A*:  $F_{(1,16)} = 0.04$ ,  $P = 0.8$ ; *B*:  $F_{(1,16)} = 0.3$ ,  $P = 0.5$ ; *C*:  $F_{(1,16)} = 0.1$ ,  $P = 0.7$ ; *D*:  $F_{(1,16)} = 0.02$ ,  $P = 0.9$ ; *E*:  $F_{(1,16)} = 0.5$ ,  $P = 0.4$ ). *B*: EMG activity during LP was similar between taste and water stimulations in the NOVEL and FAMILIAR1 sessions (ANOVA showed no main effects of stimulus or session nor interaction between the 2;  $F_{(1,353)} = 0.06$ ,  $P = 0.8$ ). See Fig. 4 for a sample of EMG activity. In contrast, mean spike count, calculated from the same experiments, increased significantly during exposure to familiar taste compared with water ( $F_{(1,24)} = 0.02$ ,  $P = 0.9$  and  $F_{(1,24)} = 6.5$ ,  $P < 0.02$  in NOVEL and FAMILIAR1 sessions, respectively; error bars are  $\pm$ SE). *C*: time course of EMG recording and neural activity in a representative session during EP and LP. A significant correlation between the EMG and neural activity was found during EP but not during LP. Data were averaged for groups of 6 trials each (last data point in each block was averaged for 5 trials). Arrows denote initiation of the 2nd experimental block during the same session.

novel tastant. We found that the GC increases its response to a familiar tastant in a distinct temporal phase of its persistent response. This increased response is not correlated with familiarity per se, but rather with familiarization to a specific experienced taste.

Although we did not monitor in detail the emergence of the signature of familiarity over time, the fact that it became apparent only at 24 h but not at 45 min after the initial encounter with the taste raises the possibility that the emergence of signature of familiarity is a slow process, which might be related to the activation of neurotransmitter receptors, modulation of gene expression, and posttranslational modifications detected in the insular cortex in the first hours after the consumption of an unfamiliar, but not a familiar tastant (Berman et al. 1998, 2000; Gutierrez et al. 2003; Koh et al. 2003; Rosenblum et al. 1997).

The rationale for our experimental protocol was based on two major observations. First, a single session of drinking a novel tastant elicits long-term taste memory (Berman et al. 2000; Bures et al. 1998). Second, consolidation of taste memory depends on neural mechanisms active over minutes to hours (e.g., Berman et al. 1998; Rosenblum et al. 1993). Hence, rats were presented with the same tastant when it was novel (NOVEL session), when it became familiar 1 day later (FAMILIAR1 session), and at 48 h (FAMILIAR2 session). This allowed distinct dissociation in time of processes that subserve memory acquisition and consolidation (NOVEL session) and memory retrieval (FAMILIAR sessions). The presentation of alternating blocks of repeated water and tastant (Fig. 1A) allowed us to ensure sufficient and continuous exposure to the stimuli, while at the same time, reducing the potential for sensory adaptation.

It is noteworthy that previous data have indicated that a few licks of a new tastant are insufficient to render that tastant familiar, as judged by the ability to form taste associations in CTA as well as to hinder association in subsequent CTA in a latent inhibition protocol; rather, it takes a substantial amount of consumed tastant, comparable to that consumed in the NOVEL session, to effectively induce long-term taste memory (Rosenblum et al. 1997; Stehberg and Dudai, unpublished data). Furthermore, both behavioral and molecular data show that it takes many minutes to several hours to establish taste familiarity (Berman et al. 1998, 2000; Rosenblum et al. 1997). We therefore assume that, when acquisition and consolidation of lasting taste familiarity are considered, one could treat the trials in the NOVEL session as repetitive exposures to a yet unfamiliar taste.

Responses in the GC are driven by converging somatosensory and chemosensory inputs arriving from the oral cavity and detected within seconds (Hanamori et al. 1998; Kosar et al. 1986; Yamamoto et al. 1989). In some studies, durations of each stimulus exposure were relatively long (often 5–20 s). This complicated the separation between somatosensory and chemosensory inputs. To allow for at least partial separation among these input variables, we restricted exposure to the stimuli to 1 s in each trial. In addition, we analyzed the response in three separated phases determined by stimulus duration and oro-facial motor activity (Figs. 3 and 4). In all our analyses, response to water stimulation was used as a reference to the response to the tastant, since we considered water to be a highly familiar stimulus. This was also evident in the stable responses to water stimulations during water sessions (Fig. 6, insets).

We first employed an analysis that considered only the units that showed significantly different response to the tastant (TRUs). This resembles a commonly employed analysis, which showed that relatively few cells responded to a specific tastant (~10–15%; Hanamori et al. 1998; Yamamoto et al. 1989; Yasoshima and Yamamoto 1998). Our results from analyzing the responses in the EP (Fig. 5) are consistent with these results. However, consistent with Katz et al. (2001), we show that the percentage of TRUs is larger when considering additional responses phases. In particular, we found 27% of TRUs when analyzing the LP of the NOVELL session.

We found that familiarity was correlated with increased activity to a tastant during the LP when the entire recorded population in the GC was considered (Fig. 6). Examination of this increased LP activity on a session by session basis (Fig. 7), suggested that specific populations participated in the processing of familiarity for specific tastants, and that the response to a familiar tastant during the LP was not a general response to familiar stimuli, but rather, correlated with the familiarization to a specific experienced tastant. Thus the percentage of activity change measured for the entire GC population should be taken as a lower bound for the “signal-to-noise” estimated for a presumed taste-specific, and perhaps familiarity-specific, neuronal group. In addition, the analysis that found no significant correlation between the familiarity response to a specific tastant and tastant specific response during the EP and MP suggests that the populations that encode familiarity of a tastant do not necessarily encode the familiarity-independent attributes of this tastant.

The increased activity during the LP in the entire population (Fig. 6) and the variability in TRUs between the phases (Fig. 5) are in accordance with reports that single neurons may process multiple types of information in the time course of their response (Lipton et al. 1999; McClurkin and Optican 1996; Sugase et al. 1999). In particular, it has been shown that single units in the GC can dynamically process chemosensory and somatosensory information along their time-varying responses (Katz et al. 2001, 2002a). Further support for this hypothesis stems from the analysis that correlated the time course of the EMG activity with neural response: a significant correlation was observed in EP only (Fig. 9C), suggesting that somatosensory inputs are processed mainly during the EP. Similar multi-modal processing phases were observed previously, although on a faster time scale (Katz et al. 2001, 2002a). The difference in time scale might result from the difference in the experimental protocol of the two studies. A proposed scheme of the dynamic processing of different variables in the GC is depicted in Fig. 10.

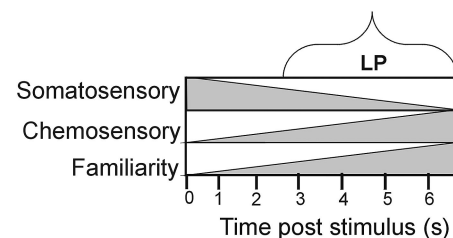


FIG. 10. Schematic representation of the different types of information processed along the temporal dimension of the GC response. The gray gradients describe relative degrees of processing of each type of information (chemosensory, somatosensory, and familiarity). Our data do not rule out the possibility that chemosensory and familiarity information are also processed during EP and MP, but are masked by the strong somatosensory input present at that time.

It could be argued that the increased response to a familiar tastant is the result of increased somatosensory input to the GC during the FAMILIAR session. Our data suggest that this is not the case. First, we used only tastants that are innately not aversive to rats; rats showed equal preference of drinking these tastants versus water on first exposure (Bahar, unpublished data). Thus we did not expect major differences in oro-facial behavior among the different sessions. This was confirmed in our video recordings, showing that typical oro-facial movements (Grill and Norgren 1978) had similar probabilities of occurrence and duration in the NOVEL and FAMILIAR sessions (Fig. 9A). Second, EMG recordings of the masseter muscle, a major facial muscle that controls jaw movements (Yamamoto et al. 1982), did not unveil differences in muscle activity between the different sessions. In contrast, neural activity recorded from rats that participated in the EMG recordings showed a significant increase in response to the familiar tastant (Fig. 9B). Third, if the increased response to a familiar tastant was indeed the result of increased motor reaction to a familiar stimulus, we would have expected an increase in neural response to all familiar tastants. However, rats tested on two different tastants showed increased response to one only (Fig. 7). This was detected in experiments using either one of the tastants, hence is unlikely to reflect familiarization with a unique taste quality. It is noteworthy that the taste cortex occupies  $\sim 4.5 \text{ mm}^2$  (Bures et al. 1998), whereas our electrode bundle captured neural responses from a volume  $\sim 500$ -fold smaller. Thus the probability of detecting familiarity signal to both tastants by recording from one bundle was inherently low. This low probability is also in line with the assumption that, in the taste cortex, stimulus properties are encoded nonhomogeneously over the cortical surface (Yamamoto et al. 1989).

What might be the physiological basis of the persistent activity observed in the GC during the LP? This activity is not the sum response of early and late response single units, since responses of single units in our recordings also contained LP (Fig. 3C; Katz et al. 2001). It is also unlikely that strong somatosensory inputs affect activity during the LP, as evident from the low oro-facial activity measured during this phase (Fig. 4); inputs that may rise from swallowing activity are also unlikely, because it was shown that the rat swallows small amounts of liquid fast (Travers and Norgren 1986). Although we cannot rule out the possibility that the LP may be affected to some degree by somatosensory inputs, we prefer the interpretation that GC persistent responses arise from interactions with other stations in the taste system (e.g., amygdala, Escobar and Bermudez-Rattoni 2000; van der Kooy et al. 1984) or reverberations of intra-cortical activity (Katz et al. 2002b). Our data show that the increased response to a familiar tastant was stimulus specific, thus the increased response during the LP could reflect retrieval of stored representation of specific familiar tastants, suggested to reside in the GC (Berman et al. 2000).

All in all, an attractive hypothesis is that networks in the GC function as associative memory networks (Hopfield 1982), which learn new tastants according to Hebbian-like rules under neuromodulatory control (Ahissar et al. 1992, 1996, 1998; Barkai and Hasselmo 1997; Ego-Stengel et al. 2001; Shulz et al. 2000). On the system's algorithmic level, is formation of taste memory triggered by taste novelty or inhibited by taste

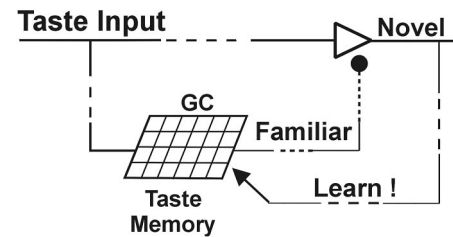


FIG. 11. A flowchart model of novelty detection in the taste system, based on findings compiled from the present as well as from earlier studies (Berman et al. 1998, 2000; Rosenblum et al. 1997). Our data suggest that Novel is the default assumption of the system for any taste input. This assumption is rejected if the taste input is recognized as FAMILIAR by an associative memory network that includes the GC. If not rejected, the NOVEL signal activates neuromodulatory systems, including the cholinergic and the noradrenergic inputs (Berman et al. 2000; Ferreira et al. 2002; Gutierrez et al. 1999) that instruct the GC network to learn the novel taste. This instruction is correlated with activation of intracellular signal transduction cascades in GC neurons, which culminates in long-term synaptic changes (Berman et al. 1998). Closed circle denotes inhibition.

familiarity (Berman et al. 2000)? Since the spontaneous and evoked activity of GC neurons is relatively low, it is tempting to assume, on the basis of our data, that the transformation of representation of novelty to familiarity is signaled by increased, rather than decreased, activity; this suggests that the cortex, as default, considers a tastant novel until proven otherwise. Hence, the formation of taste memory in the GC might be expected to be inhibited by taste familiarity (Fig. 11). This seems useful from a phylogenetic point of view, as instantaneous consumption of a novel foodstuff may prove lethal.

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