State-Dependent Modulation of Time-Varying Gustatory Responses

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INTRODUCTION

Sensory stimuli induce temporally complex responses in neural circuits (deCharms and Merzenich 1996; Friedrich and Laurent 2004; Jones et al. 2004; Nicolelis et al. 1993; Wehr and Laurent 1996). Taste is an excellent model system in which to study such responses because tastes on a vertebrate’s tongue are coded by protracted, time-varying neural responses both in gustatory brain stem regions (Di Lorenzo and Schwartzbaum 1982; Di Lorenzo and Victor 2003; Di Lorenzo et al. 2003) and in gustatory cortex (GC) (Bahar et al. 2004; Katz et al. 2001a; Kobayakawa et al. 1999). In conscious rats, in fact, GC neurons participate in multiplexing of sensory information: GC spiking responses transmit multiple types of information through time, first about the somatosensory properties of the stimulus, then about the chemical makeup of the stimulus, and finally about stimulus palatability (i.e., emotional valence) (Katz et al. 2001a).

Sensory processing is not context-free, however—it is strongly influenced by the specific states of the networks involved in coding (Aguilar and Castro-Alamancos 2005; Baizhoven et al. 1998; Erchova et al. 2002; Fanselow and Nicolelis 1999; Liu et al. 2001; Steriade 1993; Worgotter and Eysel 2000). Taste is also an ideal system with which to advance our understanding of state-dependency of processing for at least two reasons. First, we have recently shown evidence that rats undergo just such a behavioral and neural state change toward the end of a tasting session (we refer to the later, inattentive state as “disengagement”) (see Fontanini and Katz 2005), analogous to data from humans performing sensorimotor tasks (Molle et al. 2002; Slobounov et al. 2000). Second, the intrinsically emotional quality of taste stimuli allows us to probe the functional nature of state-dependent responses in a novel way: whereas network state changes have been previously shown to “gate” stimuli (e.g., Bezdudnaya et al. 2006; Murakami et al. 2005), the use of taste stimuli allows us to address the question of whether a state change modulates the actual content of temporal codes—for instance, increasing the similarity among already similar tastes and decreasing the similarity between already dissimilar tastes.

Here, we provide evidence for exactly this phenomenon in analyses of behavioral and GC taste responses recorded before and after the onset of an inattentive state. Although disengagement changed neither the overall percentage of neurons producing taste-specific responses nor the average response across neurons to tastes, it drastically changed the specifics of those taste responses. Neural responses to some tastes changed more than those to others, and our population statistics suggested that the overall effect of these changes could be described in terms of palatability: the similarity between already similar tastes (quinine and citric acid) increased, the similarity between stimuli traditionally thought of as being maximally different in palatability (quinine and sucrose) decreased, and the similarity between two already equally palatable tastes (sucrose and NaCl) remained unchanged. We interpret these results as showing that disengagement accentuates hedonic relationships, both similarities and differences.

This interpretation was confirmed by two independent converging analyses. First, the changes proved to be temporally specific as well: they occurred relatively late in the responses, at the time when palatability-related information emerges in GC responses (see Katz et al. 2001a). In addition, the neural changes were paralleled by equivalent changes in the rats’ palatability-determined behavioral responses to the tastes: disengagement decreased the hedonic similarity of behavioral responses to quinine and sucrose and increased the hedonic similarity of quinine and citric acid.

These data add to our understanding of how the information contained in neural sensory codes varies with cortical network states, showing that changes in network states not only gate stimuli but also change the content of temporal codes. Related to this, our results suggest that while primary nerves provide...
the input to central processing stations, sensory processing is strongly affected by interactions among the neurons in these central networks. Central network interactions represent a mechanism of stimulus processing beyond those described in the main theories of taste coding—the labeled-line (Scott 2004) and across-fiber pattern (Erickson 2001; Smith and St John 1999) theories—both of which focus on ascending input pathways.

METHODS

Subjects

Female Long Evans rats (250–300 g at the time of surgery) served as the subjects in this study. Animals were maintained on a 12h/12h light/dark schedule, with experiments carried out in the light portion of the cycle. Unless otherwise specified, animals in home cages had ad lib access to chow and water.

Surgery

Animals were anesthetized using an intraperitoneal injection of a ketamine, xylazine, and acepromazine cocktail (100, 5, and 1 mg/kg, respectively). Small (20–30% of induction dose) injections of ketamine were delivered to maintain depth of anesthesia. The anesthetized rat was placed on a stereotaxic frame, holes were bored in the skull for ground screws and electrode bundles, and two microelectrode assemblies were lowered to 0.6 mm above layer 5 of GC bilaterally, guided by stereotaxic measurements (AP 1.4, ML ±5 from bregma, DV –4.0 from dura). Once in position, each electrode bundle was cemented to the skull with dental acrylic as was a bolt for restraining head movements in the later experiment.

After electrode implantation, intra-oral cannulae (IOC) were inserted bilaterally—polyethylene tubes were passed from the mouth, through the masseter muscle, and out through the scalp opening (see Phillips and Norgren 1970). The scalp was then sutured, and antibiotic ointment was applied to the wound. The rat received a subcutaneous injection of penicillin (0.1 ml) immediately after surgery, and again 2 days later.

Training

Rats underwent adaptation to handling starting 2 days post surgery. Every 2/3 days, the electrodes were lowered ~70–140 μm until they were buried in a taste-responsive part of insular cortex (Katz et al. 2001a, 2002). Seven days after surgery, rats were begun on a regimen of mild water restriction: 30 min of water ad lib in the late afternoon; rats received water after training and experiments between 8 and 6 pm.

Behavior analysis

Palatability-related orofacial and ingestive behaviors were recorded throughout the experimental session via a camera mounted below the face of the rat. Three sessions—comprising a total of 209 taste deliveries (105 before and 104 during disengagement)—were randomly selected for behavioral analysis. Video segments—from 0.5 s before to 2.5 s after taste delivery—were advanced frame by frame, and purposeful mouth movements were monitored. Taste reaction times were extracted by measuring the latency of the first mouth movement after the delivery of the taste, i.e., three consecutive frames in which the mouth moved in the opening position. Two components of taste reactivity were classified (Grill and Norgren 1978a): tongue protrusions—rhythmic protrusions of the tongue on the midline beyond the plane of the incisor teeth and/or lateral protrusions extending beyond and pushing the lip forward; and gapes—large-amplitude openings of the mouth with retraction of the corners of the mouth. Each occurrence of each behavior was counted for each of the four tastes in the two conditions (pre-disengagement and disengagement). Partial or complete interruptions of the consummatory behavior—absence of mouth movements for ≥200 ms—were monitored and used to compute the portion of the 2.5 s spent performing consummatory behaviors. Video coding analysis was performed blindly of the taste delivered and the brain state.

Identification of disengagement

As described previously, the behavioral change that we refer to as disengagement is sudden and reliable: across the space of three to five trials, the rat goes from a state of task-orientation in which its lever presses are consistently and tightly related to the gaining of fluid reward to a state in which presses occur almost randomly (Fontanini and Katz 2005). Behavioral disengagement occurs simultaneously with the equally sudden emergence of high-amplitude episodes of 7 to 12 Hz μ activity in local field potentials (LFPs) records. Given their high amplitudes and identifiable characteristics, μ episodes can be detected off-line by visual inspection or auscultation and are reliably detected off-line via a simple crossing of a voltage threshold by the LFPs signal (Fontanini and Katz 2005).
Electrophysiology

Each electrode bundle included 16 25-μm wires glued to a small microdrive (Katz et al. 2001b). Recordings from all microwires, made during each session that included tastes, were amplified, band-pass filtered at 300–8,000 Hz, and digitized (Plexon, Dallas TX). Sixteen of the raw signals were also split off to a separate amplifier with filtering set for LFP recording (band-pass: 3–90 Hz), and from there to a computer, where they were digitized at 1,000 Hz.

Single neuron action potentials of >3:1 signal-to-noise ratio were isolated using a waveform template algorithm, augmented with off-line cluster cutting software (Plexon). All isolations were reliable for the entire duration of the experiment.

GC taste receptive field analysis

For each taste, all the trials from one phase (task-orientation or disengagement) were averaged. For analyses of “taste profile” (the typical measure of a taste neuron’s receptive field, see for instance Nishijo and Norgren 1997), firing rates were averaged across all the trials in each condition, and across the 2.5 s after stimulus presentation. Because we delivered multiple trials of four different tastes (with water rinses between each) to conscious rats with limited attention spans, we were unable to also deliver trials of water as a taste; therefore a neuron was defined as taste responsive if its average response to at least one taste differed significantly (P < 0.01) from its average response across all tastes (this method differs from the more typical comparison between taste response and water response, but it tests a prima facie reasonable definition of “taste-specific responses”). The significance of the difference was ascertained using a one-way ANOVA, and post hoc tests determined which taste(s) differed from the others. This test for taste-responsiveness could, in theory, underestimate the number of taste responsive neurons (e.g., broadly tuned cells that respond similarly to all stimuli but that do not respond to water may not be counted as taste responsive) and thus provides a conservative measure for taste-responsiveness.

Within-neuron quantifications of disengagement-related changes were performed by comparing two tastes × trials data arrays—one from the period of task-orientation and one from disengagement—with a two-way ANOVA (tastes × condition); neurons for which the interaction term was significant (P < 0.05) were classified as changed. Two additional control comparisons were used: to ascertain baseline levels of change due to random variability, two groups of randomly selected trials obtained during task-orientation were compared (task-oriented rand. 1 and 2). Additionally, to confirm that the putative disengagement-related changes were not simply dependent on the passage of time, trials during task-orientation were divided into “early” and “late” groups and compared (task-oriented early and task-oriented late). The significance of differences between distributions was assessed using χ² tests.

Once significant disengagement-related changes were identified, the magnitude of the change for each taste was quantified: the response during task-orientation was subtracted from the response during disengagement, and the absolute value of the result was normalized by the sum of the two responses. The significance of between-taste changes was calculated using a repeated measures one-way ANOVA; post hoc tests further evaluated any significant differences.

Principal component analysis (PCA)

PCA was performed to establish whether disengagement affected the organization of the taste space in an interpretable manner. For this analysis, each taste was described as a vector consisting of the average absolute responses across 2.5 s to that taste by each neuron whose firing rate in response to any stimulus exceeded 2 Hz (n = 60); this sample of neurons was chosen to avoid distortion that inevitably results from the inclusion of neurons with low firing rates. The robustness of the result was tested by also performing a PCA on all the neurons recorded (n = 110); qualitatively similar results were obtained when all neurons were included. The dimensions needed to represent the taste responses from the number of neurons were reduced to three on the basis of “scree plot” analysis (i.e., only dimensions before an “elbow” of variance accounted for were used, data not shown). The Cartesian distance between tastes was computed using these principal components, and the between-state differences in this distance characterized the impact of disengagement—negative differences meant increased similarity between two tastes and positive differences meant decreased similarity.

Analysis of taste response time courses

Taste temporal coding was analyzed in two stages. First, neural responses for all the neurons were divided into three time epochs, as distinguished previously: the early epoch, defined as the period between 0 and 250 ms after taste administration, has been shown to contain somatosensory information about the stimulus; the middle epoch, which extended from 250 to 1,000 ms after taste administration, has been shown to mainly contain chemosensory information relating to stimulus identity; finally, the late epoch, defined as the period after 1,000 ms after taste administration, has been shown to contain mainly palatability-specific information—that is, information about the psychological properties of the stimulus (Katz et al. 2001a). Two-way ANOVAs (tastes × condition) similar to those used for ascertaining the number of neurons changed (see preceding text) were applied for each epoch to evaluate the occurrence of disengagement-related changes. The number of units for which the interaction term was significant (P < 0.05) for each epoch was counted and plotted in Fig. 6C. As in the case of the overall response change analysis, control comparisons established the baseline likelihood of changes, and further verified that the results were not determined by the fact that the epochs were of differing lengths of time. Differences between experimental and control comparisons were assessed using a χ² test.

Next, a moving-window analysis was used to more richly characterize disengagement-related time-course changes: neural responses from all the neurons were divided into 250-ms bins (bins overlapped by 125 ms); bins were compared between the two conditions as described in the preceding text. The resulting time series of disengagement-related changes was fit with a high-order polynomial to provide a visual representation of the trend.

Histology

After the experimental sessions, subjects were deeply anesthetized, and 7 s of DC current (7 μA) were passed through selected wires. Next the rats were perfused transcardiatically with saline and fix. Sections (40 μm) were cut through the implanted areas of fixed brains, and slices were stained with Prussian blue for ferrous deposits blasted off the electrode tips, and counterstained with cresyl violet.

RESULTS

Single-neuron recordings in GC

Electrode bundles were implanted together with a small microdrive such that each rat could participate in multiple sessions. For each recording session, microwire tips were located somewhere within the deep layers of GC; when it became clear that the tips were through this region the animal was perfused, and the final resting places of the tips were marked with Prussian blue (Fig. 1A, △). Action potentials recorded from these wires were isolated using a waveform template algorithm (Fig. 1B), and isolations were checked...
using off-line cluster-cutting software (Fig. 1C) and inspection of interspike interval plots (Fig. 1D), which consistently revealed >1 ms refractory periods in the firing statistics.

Disengagement reflects a sudden reduction of attention paid to tastes

We have previously characterized the electrophysiology of disengagement as a 10-fold increase in the incidence of 7 to 12 Hz \( \mu \) rhythms in GC LFPs (Fontanini and Katz 2005). The \( \mu \) episodes are typically more than twice the amplitude of desynchronized LFPs, and appear suddenly, late in the session (Fig. 2A). Because they are so easily observed, it is not necessary to use spectral analyses to identify them—simple visual inspection of the recordings, confirmed off-line by a voltage amplitude threshold, serves to divide the first, \( \mu \)-free part of a session (which typically lasted 59 min 42 s \( \pm \) 2 min 15 s, mean \( \pm \) SE) from the later, \( \mu \)-rich part of the session (Fig. 2, A2 and B).

The sudden change in the nature of cortical LFPs in disengagement occurs simultaneously with equally sudden changes in rat behavior. We previously showed that disengagement is associated with a loss of attention to the liquid-rewarded lever press task, and that this loss of attention is not strongly associated with satiety (it may well be related to sleepiness) (see Fontanini and Katz 2005). Here, we show that disengagement is also associated with a reduction in attention paid to experimenter-delivered (i. e., not obtained through lever press) doses of sucrose (S), sodium Na, citric acid (CA), and quinine (Q).

We quantified attention to proffered stimuli in terms of simple reaction time to stimulus presentation, measured via blind examination (i. e., without knowledge of LFPs or taste) of the digital video record; many studies have specifically quantified attention in terms of reaction times to stimulus presentation (e.g., Rafal and Posner 1987), and recently this measure has specifically been used to relate attention and the spectral character of LFPs (Womelsdorf et al. 2006). Figure 2B shows that the latency of first mouth movement produced in response to experimenter-administered tastes increased by 50\% (from 420 \( \pm \) 10 to 630 \( \pm \) 10 ms, \( P < 0.05 \)) with disengagement. This result suggests that the state change in GC networks is associated with a reduction of attention to taste stimuli. For simplicity’s sake, however, and to maintain continuity with our previous work, we will continue to refer to the inattentive state as disengagement throughout this paper.

FIG. 1. Single-neuron recordings from gustatory cortex (GC). A: coronal slice from one rat with Prussian Blue spots (\( \bullet \)) marking the final resting location of the electrode wires. Wires were moved 70–140 \( \mu \)m each night after the experiment, and recordings were separated by >1 day; all 4 recording sessions took place with the electrodes in gustatory cortex (the rough boundaries of which are marked with dashed lines). B: ---, average waveform of a representative single neuron’s action potential from this experiment; - - - , confidence intervals from the template-matching algorithm. Time is on the \( x \) axis and voltage is on the \( y \) axis (horizontal line is 0 V). C: plot of principal components 1 and 2 from the off-line cluster-cutting software (scatterplots of other parameter pairs gave qualitatively similar results), showing several clouds of dots, each of which represents a single possible action potential exemplar. Individual exemplars of the single neuron action potential displayed to the left form a clearly dissociated cluster (\( \bullet \)). D: interspike interval plot of this same single neuron (time since previous spike on the \( x \) axis, number of action potentials—or “counts”—on the \( y \) axis), showing the appropriate gamma distribution shape and refractory period.

FIG. 2. Identification of disengagement, a change of coherent cortical state associated with behavioral changes. A1: representative ~120-min trace of GC local field potentials, showing the time course of the occurrence of 7 to 12Hz activity characteristic of disengagement. On average, 7 to 12 Hz activity appeared 60 min after the beginning of the experiment. A2: detail of LFPs characteristic of task-orientation (top) and disengagement (bottom). B: collapsing across subjects and sessions, the percentage of time per second (\( y \) axis) in which GC LFPs showed \( \mu \) rhythms was >10-fold higher after the 1st hour from the beginning of the experimental session than before. The reaction time of orofacial ingestive behaviors (onset of the 1st mouth movements) parallels the amount of 7 to 12 Hz oscillation—during disengagement it is significantly longer than during task-orientation (see text for details).
Disengagement did not change overall prevalence of taste responses

We first characterized GC taste responses in terms of overall magnitude, averaged across at least 7 trials and collapsed across 2.5 s of poststimulus time. Across 12 recording sessions, 110 GC neurons were isolated (average = 9.2, 6–12 per session) in three conscious rats (36 from rat 1, 35 from rat 2, and 39 from rat 3). The average spontaneous firing rate across all recorded neurons was 4.9 ± 0.7 Hz before disengagement and 3.2 ± 0.3 Hz during disengagement. In a paired t-test, this difference was significant (n = 110; P < 0.05). The disengagement-related change in baseline firing rates tended to inflate the significance of changes to be discussed in the following text—similar taste responses before and after the state change often appeared different when normalized to differing levels of spontaneous firing. To ensure that our estimates of the prevalence of response changes were conservative, therefore, we chose to use absolute firing rates in our analyses rather than responses normalized to prestimulus activity.

Of 110 GC neurons, 44 (40%) responded in a taste-specific manner (i.e., a 1-way ANOVA for tastes resulted in a P < 0.01) during the task-oriented phase of the sessions (Fig. 3A, left). The situation was almost identical during disengagement (Fig. 3A, right), when 42 GC neurons (38.2%) were taste-specific. Note that because taste delivery consistently halted μ (Fontanini and Katz 2005; see also Pfurtscheller et al. 1997; Tiihonen et al. 1989), taste responses in both phases of the session were produced in the context of similarly desynchronized LFPs. The taste-responsive neurons broke down into almost identical distributions of “Na-”, “S-”, “CA-”, and “Q-best” in the two parts of the sessions (Fig. 3B)—the nominal order of prevalence in both phases was CA > Na- > Q > S, but, averaged across the sample, GC was similarly responsive to all tastes in both parts of the session (Fig. 3C). Clearly, disengagement does not reflect a reduction in overall cortical responsiveness to tastes.

Disengagement changed taste receptive fields

While the overall percentage of neurons showing taste-specific responses was not altered by disengagement (χ² test P > 0.5), the specific taste profiles of many neurons did change. Figure 4A shows such changes in three simultaneously recorded GC neurons. The first of these neurons, which did not respond in a taste-specific manner during the task-oriented phase, gained a substantial Q response with disengagement. The second cell, a narrowly tuned “Q-best” neuron prior to disengagement, became a less narrowly tuned “Q/CA-best” responder afterward. Finally, the third simultaneously recorded neuron was stable across the state change, producing identical responses during task-oriented and disengaged portions of the session.

Across the entire sample of 110 GC neurons, a two-way ANOVA [taste × condition (task-orientation or disengagement)] revealed that 46 neurons (41.8%) changed with disengagement (Fig. 4C). Of these 46 neurons, 12 (10.9%) of all units, 26.1% of the units that changed, 27.3% of all taste units that were taste-responsive before disengagement were not afterward, and 9 (8.2% of all units, 19.6% of changed units and 21.4% of taste units in disengagement) became taste-specific only after disengagement. Finally, 16 neurons (14.5% of all units, 34.8% of units that changed and 36.4% of taste units) were taste-responsive in both parts of the session but changed their responses with disengagement, and 9 (8.2% of all neurons and 19.6% of changed neurons) remained taste-unresponsive but changed absolute levels of responding. Of this former group of 16 neurons, 9 (56.3%) changed their taste profiles with disengagement—responding best to another taste or acquiring or losing best responses to other tastes—whereas 7 (43.7%) maintained the same taste preference.

We performed two control analyses to confirm that 41.8% of the recorded neurons truly changed their response profiles with disengagement. First, we ascertained how many changes might have occurred by chance by randomly sorting trials from the task-oriented portion of sessions into two groups and comparing taste profiles between those groups. As shown in Fig. 4C (task-oriented rand. 1 vs. task-oriented rand. 2), only 4% of the neurons had different response profiles across these two arbitrary groups of trials according to two-way ANOVAs. Next, we compared trials from the first half of the session to trials from the second half of the rats’ task-oriented period (i.e., from just prior to disengagement). The third bar in Fig. 4C (task-oriented early vs. task-oriented late) shows that these two sets of trials were also very similar—only 9 (9.1%) GC neurons changed across this interval, which covered on average an hour of recording (Fontanini and Katz 2005). This number is not significantly different from that observed by chance (χ² P > 0.05). We conclude that the observed changes...
were not caused by mere passage of time, and are in fact specifically disengagement-related.

Disengagement-related neural changes can be interpreted in terms of palatability

Examination of the response profiles in Fig. 4, A and B, suggests the possibility that disengagement did not change responses to each of our four taste stimuli monolithically; for example, Q responses seemed the most strongly affected in neurons 1 and 2. To test whether this observation was correct—that is, whether disengagement changed certain responses more than others—we first quantified how disengagement affected the coding of each taste within the sample of neurons that changed their responses with disengagement. The results of this analysis are shown in Fig. 4D, which reveals that disengagement affected all four tastes but that responses to Q and Na were slightly more plastic than those to CA and S. The exact order of effect size was Q > Na > S > CA; this order was unrelated to the order of overall stimulus effectiveness (Fig. 3C). When the analysis was restricted to neurons with response frequencies >2 Hz, the changes in Q and Na responses were significantly (P < 0.05) larger than those to CA and S.

Inattentive animals need to remain responsive to “important” stimuli for survival’s sake (e.g., Bezdudnaya et al. 2006; e.g., Usher et al. 1999); this fact led us to hypothesize that specific disengagement-related changes might be interpretable in terms of the relationships between important, highly valenced tastes. To test this possibility, we performed a simple multivariate analysis of how disengagement affects ensemble coding of tastes, focusing on the relationship between most palatable and unpalatable tastes in our array (S and Q), and on the relationships between the most similar tastes in our array (in the case of S, this taste was Na; in the case of Q, this taste was CA). A PCA of ensemble taste responses allowed us to quantify stimulus discriminability as a function of the linear distance (in arbitrary units) between stimuli in a principal component taste space. Changes in discriminability wrought by disengagement could therefore be summarized in terms of changes in that distance function—negative changes meaning that the two tastes have moved closer together (i.e., their neural responses have become more similar in disengagement) and positive changes meaning that the two tastes have moved farther apart (i.e., their neural responses have become more distinct in disengagement).

Figure 5A shows the basic result of this analysis using all the GC neurons for which response frequencies were >2 Hz (this subset of 60 neurons provided the most stable solution, by eliminating distortions inevitably introduced by lower firing rates; the robustness of the result was confirmed by similar results in a PCA based on all 110 the neurons). After disengagement, Q, the most aversive taste in our array, became more distinct from both Na and S, the palatable tastes in our array, going from a distance of 3.8 arbitrary units (a.u.) during
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Figure 5B presents two-dimensional projections of the three-dimensional solution (PC1 was largely uninformative, but the results described in the preceding text were based on the full 3-dimensional solution), in which these results can be seen graphically. The ensemble codes for each taste are shown separately for responses gathered during task-orientation (S, Q, CA, Na) and disengagement (S’, Q’, CA’, Na’), with solid arrows showing the disengagement-related movement of each taste through space; dashed lines in the left and right panels show the path of movement that one taste (S in the left panel, CA in the right) would follow if moving directly away or toward Q. S moves almost directly away from Q (left) during disengagement, at an angle of 171° to the line between the 2 tastes. S and Na move in parallel (middle), CA moves directly toward Q (right), at an angle of 4.5° to the direct line between the two tastes. C: analysis of orofacial behaviors during task-orientation and disengagement. The ratio between S and Q for the average number of tongue protrusions per trial increased with disengagement (left), suggesting that S and Q became more distinct after disengagement. Meanwhile, the ratio between S and Na changed very little (note that the y axis goes only to 1.4 in this panel). Finally, the ratio between Q and CA for the average number of gapes per trial decreased after disengagement (right), supporting an increased similarity between aversive tastes.

Analyses of rat behavior confirm the palatability-specific nature of disengagement-related changes

The electrophysiological results described in the preceding text suggest that a reorganization of the taste space occurs with disengagement, such that Q and CA become more similar, Q and S become less similar, and the similarity between Na and S (tastes with already identical palatabilities) remains constant. These results must be regarded as inconclusive, however, unless they can be related to behavior. We therefore analyzed salient aspects of the videotaped behavioral responses to ad-
ministered tastes—taste reactivity and the duration of ingestive responses (Breslin et al. 1992; Grill and Norgren 1978a)—to test whether changes similar to those described above could be observed in the rats’ behavior. We focused on relationships involving Q because Q is the prototypical aversive stimulus with a palatability vastly different from that of S and similar to that of CA (particularly in situations related to safety, such as are hypothesized to be particularly important during disengagement, see Nowlis et al. 1980). In the following text, we report that, as expected, disengagement increases the difference between behaviorally determined palatability of Q and S, while decreasing the difference between the palatability of Q and CA and leaving the differences between the palatability of S and Na unchanged.

Taste reactivity communicates the palatability of the consumed taste (Berridge 2000) and was therefore the behavior analyzed for this experiment. Lateral tongue protrusions and other licks are behaviors that indicate an animal finds a taste palatable, while gapes (wide yawning openings of the mouth) indicate that a taste is aversive as does a general failure to perform ingestive behaviors (mouth movements). Although all tastes induce some mix of these behaviors—in particular, most tastes induce at least some licks in water-restricted rats such as ours—Q causes significantly (P < 0.05) fewer licks than S (Q: 3.8 ± 0.6; S: 8.1 ± 0.5; measured before disengagement), and both Q and CA cause more gapes than S (Q: 1.4 ± 0.2; CA: 0.4 ± 0.02; S: 0; again measured before disengagement).

To quantify the effect of disengagement on palatability, we calculated ratios between palatability-driven behaviors for pairs of tastes (the use of ratios allowed us to compare the similarities of tastes independent of any overall changes in the frequencies of behaviors caused by disengagement). Disengagement changed these ratios in ways that mirrored the neural results: the ratio of licks to S and Q grew from 2.1 (twice as many licks for S) during the task-oriented phase of sessions to 18.6 (almost 20 S licks for every lick to Q) during disengagement (Fig. 5C, left). This nearly ninefold increase in the difference between the palatability of the two tastes is almost entirely accounted for by the precipitous reduction of licks to Q. The ratio of licks between S and Na is almost entirely unchanged by disengagement, meanwhile, the ratio is 1 before disengagement and 1.2 during disengagement, a change of only 20% (Fig. 5C, middle; note the scaling of the y axis).

The increasing similarity between the two aversive tastes, meanwhile, was visible in disengagement-related changes in gaping (the most common measure of taste aversiveness). The ratio of gaping to Q and CA went from 3.2 (many more gapes to Q than CA) during the task-oriented period to 1.2 (almost exactly the same number of gapes to each) during disengagement (Fig. 5C, right). Disengagement both increased the number of gapes to CA and decreased the number of gapes to Q; although this change appears at first blush to reflect an overall reduction in aversiveness in Q, it is worth noting that another measure of aversiveness—the number of trials in which rats made short lasting (~0.5 s) mouth movements in response to taste administration—increased for both CA and Q with disengagement. Whatever the raw valence of each taste, these data suggest, consistent with the above-discussed neural results, that disengagement is associated with a ninefold decrease in the hedonic similarity between S and Q, no change in the hedonic similarity between S and Na, and a threefold decrease in the hedonic difference between CA and Q.

Analysis of temporal coding also suggests that disengagement-related changes are palatability-specific

Again, our hypothesis, based on averaged GC responses and confirmed by behavioral analysis, is that disengagement is associated with changes in the processing of basic tastes. In both cases, the changes can be interpreted as palatability-specific: disengagement seems to cause an increase in hedonic similarity between tastes with already related palatability and a decrease in hedonic similarity between tastes with distinct palatabilities.

On the basis of our previous work on taste temporal coding, one further independent test of the preceding interpretation was developed. We previously demonstrated that palatability-related information does not emerge in GC temporal codes until several hundred milliseconds after the taste hits the tongue (Katz et al. 2001a); we reasoned, therefore, that if the electrophysiological changes caused by disengagement are truly palatability-related, then they too should specifically appear late in the responses. A comparison of peristimulus time histograms (PSTHs) made before and after disengagement, such as that presented in Fig. 6, provides evidence in favor of this prediction. For the representative single neurons shown in Fig. 6, A and B, the task-oriented taste responses (the black PSTHs) differed from the disengaged taste responses (the transparent green PSTHs overlaying the black) primarily in the latter half of the first 2.5 s; this difference is made more clear in the panels below the overlain PSTHs, which shows the difference between the two conditions. For the neuron displayed in Fig. 6A, the late Q response became much weaker following disengagement, while for the neuron in Fig. 6B, the late responses to S, Q, and CA all changed.

We quantified our test of the temporal coding hypothesis at the population level (i.e., using the entire 110-neuron sample) in two ways. First, we divided the initial 2.5 s of our GC taste responses into three periods, similar to those described previously (Katz et al. 2001a) (see METHODS), and re-evaluated the percentage of neural responses that changed with disengagement. The results of this analysis are presented in the dark set of bars in Fig. 6C. Early GC responses proved almost completely stable, only 5.4% of the sample changed this part of their responses. A significantly larger number of neurons (24.5%, χ²pearly-middle < 0.01, n = 110) underwent changes in the second period, but by far the largest number of disengagement-related change occurred late in the responses, (39.1%, χ²Pmiddle-late < 0.05, n = 110), demonstrating that disengagement-related changes are heavily (although not completely) biased to occur late in GC responses. Results for each taste, examined individually, were qualitatively similar (data not shown).

We repeated our two control comparisons—comparing early and late task-oriented trials, and comparing one random subset of trials to a second, independent random subset of trials (again within task-oriented periods)—with the data broken down into the three epochs. The results of these analyses are shown in the gray and white bars of Fig. 6C, respectively. There was no significant tendency of the changes to occur late in either of the control comparisons. Thus we conclude that the late nature of the disengagement-related changes was not an artifact of the analysis.
While disengagement-related changes clustered, as expected, late in the GC responses, some did occur in the middle of the three epochs analyzed here—earlier than previously expected. This is not surprising, as our previous experiments, however, had only roughly identified the time at which palatability emerges (see Fig. 6 in Katz et al. 2001a). Furthermore, the coarseness of the epochal analysis leaves open the very real possibility that changes were not randomly scattered throughout the middle epoch but rather clustered very close to the beginning of the late epoch.

To test this possibility, we performed a fine-grained moving-window analysis of the change data. This analysis is summarized in Fig. 6D: a fifth-order polynomial smooths the data. A look at either the polynomial fit or the points underlying it makes it clear that the “middle” epoch changes actually occurred quite late: disengagement produced almost no changes in the first 625–650 ms of GC taste responses. The number of changes increased dramatically near the 1-s mark; after this, the prevalence of changes stabilized at a level much higher than that present during the earlier portion of the responses (they then rose to a second peak after 2-s post stimulus delivery). This analysis confirms that even the earliest disengagement-related changes were, in fact, closely associated with the late aspects of the GC responses and that they therefore were directly related to palatability as predicted by the neural coding and behavioral results.

DISCUSSION

Conscious rats involved in a tasting session eventually undergo disengagement—a nonlinear behavioral state change characterized by a loss of interest in the task and task-related stimuli. This state, which probably reflects the action of multiple processes including impatience and sleepiness (but that does not appear to reflect satiation) (see Fontanini and Katz 2005), is associated with a dramatic increase in sensory cortical network oscillations in the 7 to 12 Hz range, analogous to those observed during poor task performance by human subjects (Molle et al. 2002; Slobounov et al. 2000).

Here we extend our previous results, first by showing that disengagement reflects a reduction in overall attention to taste stimuli, measured as an increase in initial behavioral reaction times to tastes (see Womelsdorf et al. 2006). More centrally, we show that behavioral and neural responses to tastes delivered to disengaged rats differ extensively from pre-disengagement responses. Three independent analyses all converge on the conclusion that these differences are palatability-specific: disengagement decreases the similarity between neural responses to Q and S (and Na), while increasing the similarity between neural responses to CA and Q and leaving the similarity of Na and S unchanged; disengagement-related changes in palatability-driven behavioral responses parallel the neural changes; and the neural changes wrought by disengagement occur almost exclusively late in GC responses, at the time that...
palatability-specific information is known to emerge in taste temporal codes.

Disengagement does not change the overall number of taste specific neurons, nor does it change the percentage of neurons sensitive to each of the four basic tastes. Thus the emergence of cortical μ rhythms in the gustatory cortex does not result in a simple gating of stimuli as has been described previously for other sensory systems (Bezdudnaya et al. 2006; Engel et al. 2001; Fanselow and Nicolelis 1999; Fries et al. 2001; Kakigi et al. 2003; Luck et al. 1997; Massimini et al. 2005; Reynolds and Chelazzi 2004; Roelfsema et al. 1998). Rather disengagement changes the processing of tastes in a subtle and meaningful way: while response likelihood remains stable across network changes, some neurons lose, some acquire, and some change their taste specificity. In the case of gustatory responses, of which palatability is an intrinsic component, disengagement is associated with a rearrangement of ensemble activity, such that some taste responses become less discriminable and some become more discriminable—those with palatabilities that are already similar become still more similar, and those with very different palatabilities become still more different.

It is worth noting that these results come by virtue of analyses that require the delivery of multiple trials of each taste to conscious rats. A dataset of one to two trials per taste (the norm in taste research) would not have provided the statistical power necessary for these analyses and in fact would have missed all but the few most intense responses. To ensure that we could collect the necessary number of trials per taste, however, we sacrificed the size of the taste battery. It is likely that adding additional sweet or bitter tastes to our array would have merely replicated results shown for sucrose and quinine, however. As with sucrose and NaCl, a set of sweet (or of bitter) tastes would start at a near ceiling in similarity and would thus not have increased in similarity with disengagement.

We previously showed (Katz et al. 2001a) that GC neurons participate in a feat known as multiplexing, transmitting multiple types of information through time; information about stimulus palatability was demonstrated to emerge relatively late in the taste responses (for examples of analogous processes in other sensory systems, see Friedrich and Laurent 2001; Friedrich et al. 2004; Sugase et al. 1999). Our prediction here, borne out by the data, was that disengagement-related changes in palatability would therefore be found specifically in this later part of the neural taste responses. In fact, our data show that the earliest changes occur well into the time courses of the neural responses, and only a few hundred milliseconds earlier than our previous rough estimates had led us to expect (a fact that can be accounted for by improvements in our stimulus delivery and analysis techniques). This observation further strengthens the conclusion that changes induced by disengagement occur along the palatability axis. The fact that the earliest disengagement-related neural changes appeared at a similar latency to the increased taste reaction times is a further support to the notion that palatability is represented in only a portion of the time course of the response.

While we cannot conclusively rule out the possibility that palatability-related GC activity constitutes “efference copy” (Andersen et al. 1997) of brain stem palatability processing (Grill and Norgren 1978b; Travers et al. 1987)—we cannot conclusively rule out peripheral contributions (changes in tongue position with disengagement, perhaps) to our results, in fact—our data together with existing literature suggest that some of the work of taste is done in networks that include the forebrain. Although basic palatability-related behaviors can be induced in a decerebrate preparation (Grill and Norgren 1978b), these behaviors are abnormal (Flynn and Grill 1988; Grill and Norgren 1978b); in fact, palatability processing can be altered by relatively small lesions restricted to taste thalamus, cortex, hypothalamus, or amygdala (Flynn et al. 1991; Galaverna et al. 1993; Grill and Norgren 1978b; Touzani et al. 1997). It appears highly likely that cortex is involved in processing palatability.

Conclusion

This work demonstrates that sensory coding is influenced by brain states and not simply by the specifics of the pathways carrying sensory information to the brain. One major implication of our findings is specific to taste, and another is general to sensory processing. First, our data suggest that part of taste processing involves mechanisms beyond those described by the “labeled-line” and “across-fiber pattern” theories of taste (Scott 2004; Smith and St John 1999). Both of these theories primarily describe the input to taste-processing systems, but our data suggest that much of the work of taste processing is performed by the neural networks handling the stimulus once it arrives and is therefore a function of the specific state of that network. More generally, the inattention associated with the appearance of μ oscillations (Fontanini and Katz 2005; Molle et al. 2002; Slobounov et al. 2000; Womelsdorf et al. 2006) does more than gate of stimuli (e.g., Bezdudnaya et al. 2006; Murakami et al. 2005): disengagement simplifies the stimulus space in a way that makes excellent sense for an animal that needs to remain responsive, even when inattentive, to important environmental stimuli (see also Usher et al. 1999).

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REFERENCES


