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Gustatory and Anticipatory Signals in the Gustatory Thalamocortical Pathway of Alert Rats

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Abstract of the Dissertation

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Neurons in the gustatory cortex (GC) encode chemosensory information, palatability and expectation. The representation of these variables is believed to result from the integration of thalamic and limbic inputs. While the limbic-cortical pathway is well studied, very little is known about the gustatory thalamus (VPMpc).

To understand the role of VPMpc in taste processing, in the first portion of this dissertation, multi-electrode electrophysiology in rats engaged in a general expectation task was employed. Rats were trained to self-administer tastants with a nose-port entering following an auditory cue. During the inter-trial interval, rats also received un-cued, passive deliveries. VPMpc neurons exhibited time-varying taste responses, which encoded both chemical identity and taste palatability. Comparing cued and un-cued, taste-evoked responses revealed that expectation improved taste coding. Neurons in VPMpc also responded to the auditory cue. Control experiments demonstrated that the cue responses were neither driven by sensory nor motor aspects of the task. Moreover, relating cue responses to behavior showed that the strength of cue responses depended on the attentive state of the animal and predicted the animals' behavioral reaction to the cue (respond or ignore). These results provide the first description of how the VPMpc of alert rats encodes gustatory and anticipatory information according to the state of the animal.

Although cue responses in the taste system suggest the processing of anticipation, whether cue-related activity is important in driving anticipatory behavior is unknown. To address this issue, in the second portion of this dissertation, a Pavlovian conditioning task was developed. After learning to associate a cue to a food pellet delivery in a port, mice showed an

increase of port entries during the cue. Neurons in the mouse GC showed significant modulations during cue presentation. Inactivating GC, by drug infusion or optogenetics, impaired the conditioned port-entering behavior. These results demonstrate that the cue-induced activity in mouse GC is necessary for the expression of the cue-induced behavior.

Altogether, this dissertation extends the role of VPMpc to the coding of expectation and demonstrates the behavioral significance of anticipatory signals in the gustatory system.

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Chapter 1

General Introduction

The gustatory system is devoted to detecting and analyzing information about food and beverages. Its main function is to encode information about the chemical properties of food and to translate that into gustatory percepts. Sugars are perceived as sweet, salts as salty, acids as sour, alkaloids as bitter, and amino acids as umami (savory). Differences in concentration are translated by the gustatory system into differences in taste intensity. In addition to encoding taste qualities, neurons in the gustatory system also integrate tactile and thermal information from the somatosensory system (Carleton et al. 2010).

The gustatory system does more than processing the physiochemical properties of taste. It also encodes the affective value of food and beverages. Palatability, i.e. the liking or disliking associated with taste, is also processed by the gustatory system (Yamamoto 1984; Yamamoto et al. 1989; Breslin et al. 1992; Travers & Norgren 1986; Soderpalm & Berridge 2000). For instance, it has been shown that a subset of gustatory neurons tend to respond similarly to tastants with similar palatability (Katz et al. 2001; Fontanini & Katz 2006; Sadacca et al. 2012; Li et al. 2013; Piette et al. 2012; Jezzini et al. 2013; Fontanini et al. 2009) and damaging the gustatory system impairs palatability related behaviors and percepts (Berridge 2000).

Recent work has shown that the gustatory system is also involved in encoding expectations associated with taste (Saddoris et al. 2009; Gardner & Fontanini 2014; Nitschke et al. 2006; Veldhuizen et al. 2011). In natural contexts, tasting is typically the final act of consummatory behaviors, which are initiated by some anticipatory stimuli. The anticipatory signal can evoke expectations of food. For example, the intense aroma of coffee and the sight of a small cup containing a black, dense fluid may lead you to expect the taste of an espresso. These preformed expectations, which can influence how we perceive taste and our dietary decisions, are encoded in the gustatory system.

Thus, the ability to integrate sensory, affective and anticipatory information makes the gustatory system an excellent model for studying multimodal information processing in early sensory pathways. Over the past years, most of the studies on the integration of these signals have focused on the gustatory cortex (GC) of alert animals. These studies have been instrumental in showing that GC neurons not only encode taste quality, somatosensation, and palatability, but also encode taste expectation (Katz et al. 2001; Sadacca et al. 2012; Samuelsen et al. 2013; Samuelsen et al. 2012; Gardner & Fontanini 2014; Jezzini et al. 2013; Stapleton et al. 2006; Gutierrez et al. 2010; MacDonald et al. 2009; Fontanini & Katz 2006; Grossman et al. 2008). While we have learned much from those GC-focused studies, other key areas of the gustatory

system remain poorly understand. For instance, very little is known about the gustatory thalamus, which provides information to GC (Kosar et al. 1986; Holtz et al. 2014; Ogawa & Nomura 1988). Recently, the gustatory thalamus has been implicated in complex, taste-guided behaviors (Reilly 1998). However, electrophysiological recordings in alert animals are limited. Therefore, in this dissertation, we have performed a series of experiments to investigate information processing in the gustatory thalamus.

In this Chapter, I will first introduce the organization of the gustatory system and outline classical theories on taste coding. Second, I will review recent progresses on information processing in the gustatory cortex and the development of novel theories on taste coding and information integration in GC. Third, I will review evidence revealing the limits of current models on taste processing. Finally, I will explain the rationale behind our experimental design and briefly introduce the contents of this dissertation.

1.1. Gustatory System and Taste Coding

1.1.1. Anatomical Organization of Primary Gustatory Pathway

The mammalian peripheral sensory unit for taste is the taste bud. Taste buds are distributed on the tongue and in the entire oral cavity. Each taste bud contains three morphologically distinct cell types (glia-like cells [type I], taste receptor cells [type II], and presynaptic cells [type III]) as well as afferent nerve fibers (Carleton et al. 2010; Chaudhari & Roper 2010; Finger 2005). Different taste receptors have been identified to detect different chemicals (Chandrashekar et al. 2010; Chandrashekar et al. 2006; Chandrashekar et al. 2009; Pérez et al. 2002; Huang et al. 2006; Vandenbeuch et al. 2008; Shigemura et al. 2008). Although each receptor cell expresses only one type of taste receptors (Carleton et al. 2010), each taste bud contains multiple types of receptor cells (Chaudhari 2014; Chaudhari & Roper 2010). All three types of cells transduce taste information and communicate across them using neurotransmitters such as adenosine triphosphate (ATP) and serotonin (5-HT). After these complex interactions within taste buds (Liman et al. 2014; Chaudhari & Roper 2010; Chaudhari 2014), taste information is carried by cranial nerves into the central nervous system.

As shown in Figure 1.1, three cranial nerves (VII facial, IX glossopharyngeal, and X vagus) are responsible for conveying taste information to the rostral part of the nucleus of the solitary tract (rNST) in the medulla (Norgren & Lundy 1995; Beckstead & Norgren 1979). The trigeminal nerve (V), which conveys tactile information, also converges in the rNST and modulates taste information. Ascending fibers from rNST project ipsilaterally to the parabrachial nucleus (PBN) in the pons in rodents, while they project directly to the thalamus and to limbic areas in primates (Beckstead et al. 1980). Ascending projections from PBN diverge into two pathways. First, PBN projects to the parvicellular part of the ventroposterior medial nucleus of the thalamus (VPMpc) bilaterally (with a heavy ipsilateral bias). Second, it also has distributed, ipsilateral projections to the ventral forebrain limbic system, including the bed nucleus of stria terminalis, the lateral hypothalamus (LH), and the amygdala (both the basolateral amygdala [BLA] and the central amygdala [CeA]) (Norgren 1974; Yamamoto 2006; Tokita et al. 2007;

Fulwiler & Saper 1984). Direct projections from PBN to the gustatory cortex have also been documented, but are believed to play only a minor role (Saper 1982). From VPMpc and from limbic areas, afferent fibers converge in the gustatory cortex (GC) located within the insular cortex (IC). GC projects to a wide range of areas including the orbital frontal cortex (OFC), the medial prefrontal cortex (mPFC), the striatum, the amygdala (Maffei et al. 2012). Higher-order taste areas (e.g., GC and BLA) also send projections back to the brain stem (Lundy & Norgren 2001; Smith & Li 2000).

Over the past years, the chemosensory research community has focused on the understanding of taste processing in various nuclei along the gustatory pathway. Historically, GC has been the most studied structure in alert animals. For this reason and for its relationship with VPMpc, I will focus the introduction of this dissertation on reviewing studies in GC, in order to illustrate taste coding theories and recent developments.

1.1.2. Classical Theories of Taste Coding in GC

The most studied aspect of taste processing is the coding of chemical identity, or taste quality. There are two classic theories on how the chemical identity of taste is encoded. The first is called the "labeled line" theory (Frank et al. 1988; Pfaffmann 1955; Pfaffmann 1974; Pfaffmann et al. 1976; Pfaffmann et al. 1979; Scott & Giza 1990). It hypothesizes that specific sets of neurons (both at the periphery and in the central nervous system) respond robustly to only one given taste quality and carry the totality of its information. Neurons belonging to different taste qualities are segregated along the taste system (Figure 1.2A). The second theory is named the "across fiber pattern" theory (Erickson 1968; Erickson 1963; Erickson et al. 1980). It asserts that taste quality is encoded in the activity of ensembles of neurons and the information is distributed among the ensembles. A given neuron is activated by different taste qualities with different response magnitudes; and a given taste is represented by the activated pattern or "landscape" of the neural ensembles (Figure 1.2B). Therefore, the first theory predicts that neurons respond only to single tastants, or are narrowly tuned, and form segregated pathways. These predictions suggest (but do not require) the existence of a chemotopic map with different taste quality separate from each other. On the contrary, the second theory predicts that neurons respond to multiple tastants, or are broadly tuned. This theory does not rely upon a chemotopic map in which all the taste qualities are segregated and non-overlapping.

Evidence for both coding schemes has been observed in GC from studies using different methodologies. Electrophysiological recordings of single neuron activity provide the ability to assess neuronal representations of taste and have been widely used to study neural coding. Both narrowly tuned and broadly tuned neurons in either anesthetized or alert animals have been reported (Yamamoto 1984; Yamamoto et al. 1985a; Yamamoto et al. 1989; Hanamori et al. 1998; Katz et al. 2001; Stapleton et al. 2006; Jezzini et al. 2013). Reconstructions of recording sites have been used to test the "chemotopic map" hypothesis (Yamamoto 1984; Yamamoto et al. 1985a). Although the majority of neurons recorded were broadly tuned in these studies, the authors analyzed recording locations by partitioning GC into 16 equal-sized rectangles and sorted neurons according to their "best-taste" (the taste stimulus that evoked largest response). They found that "best-taste" neurons have a trend to cluster spatially. The sweet-best neurons were biased to anterodorsal regions, while the bitter-best to the posterior region. Salt responses

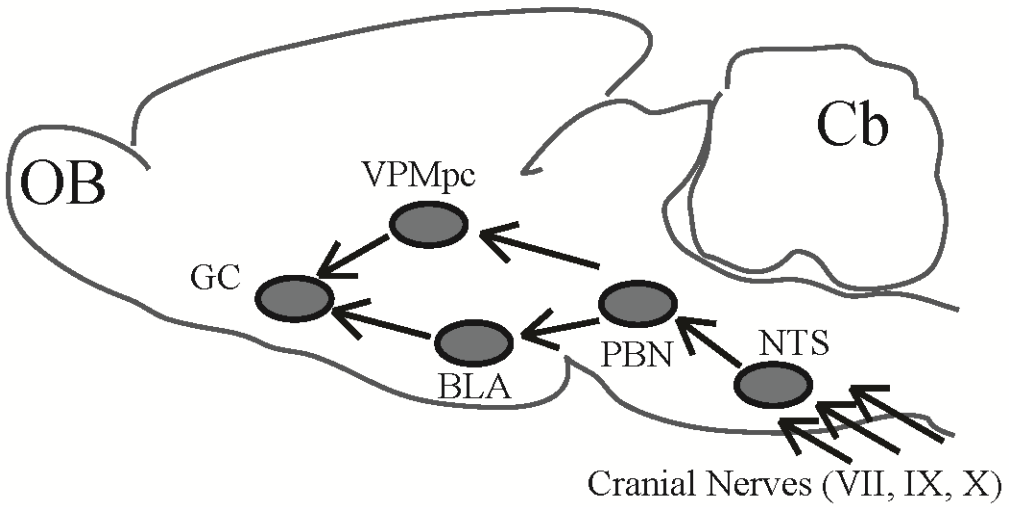


Figure 1.1: Taste pathways in the rodent brain. Three cranial nerves (VII, IX, X) innervate different parts of the oral cavity and convey taste information to the rostral part of the nucleus solitory tract (NTS). NTS projects to the parabrachial nucleus (PBN) in the pons. PBN projects to both thalamus and limbic regions. Both the parvicellular portion of the ventral posteriomedial nucleus of thalamus (VPMpc) and basolateral amygdala (BLA) project to the gustatory cortex (GC), from which taste information goes to other brain areas, such as orbitofrontal cortex, nucleus accumbens, and hypothalamus (not shown). Feedback projections, known to be present at multiple levels, are not shown.

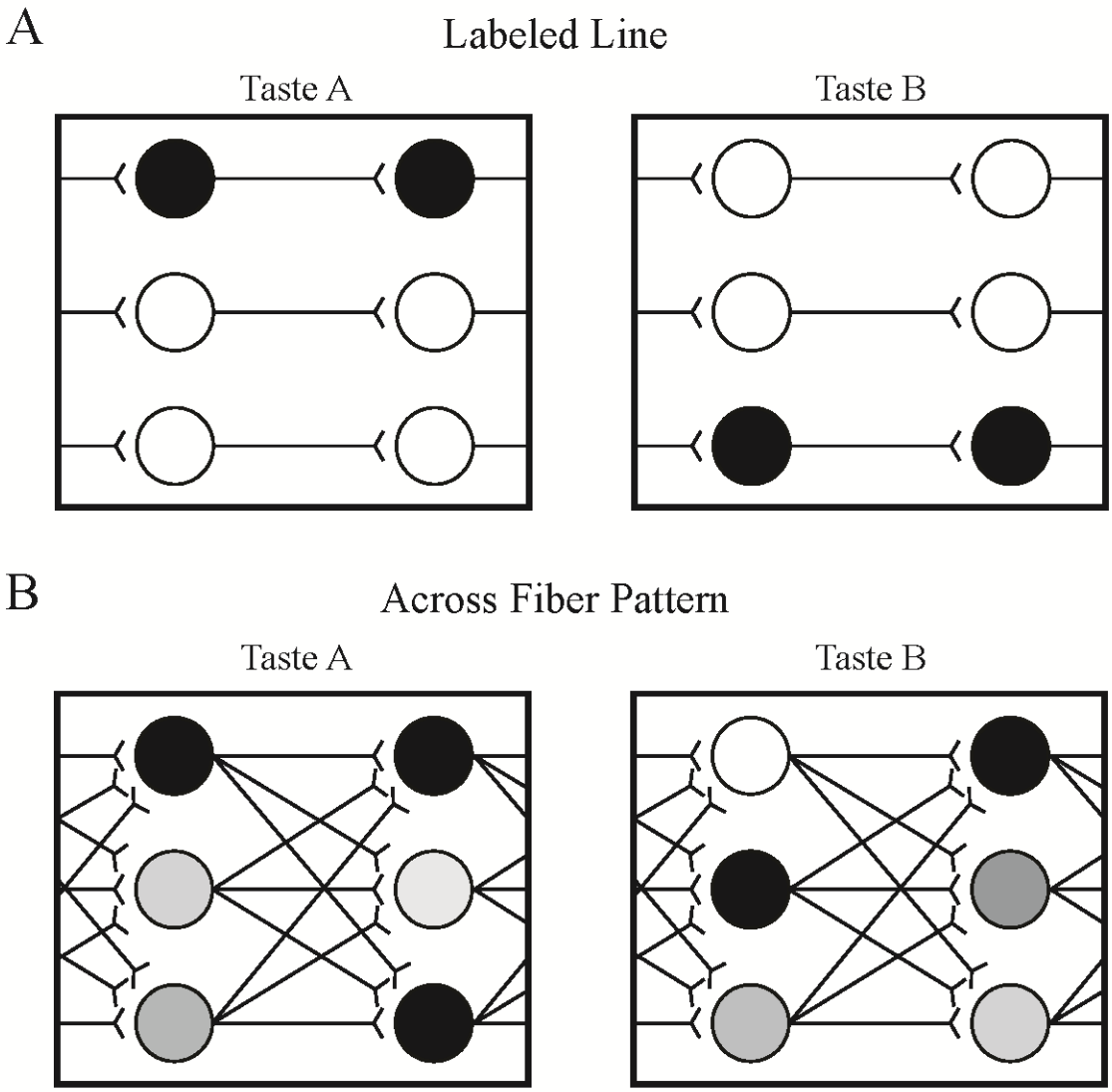


Figure 1.2: Schematic representation of two classical theories on taste coding: labeled line and across fiber pattern. Circles represent neurons and the filling represents the magnitude of neural responses (black: maximal, white: minimal). Left and right pannels represent the activity evoked by taste A and B, respectively. A. The labeled line theory. Neurons respond robustly to only one given taste quality (i.e., narrowly tuned) and carry the totality of its information. Neurons belonging to different taste qualities are segregated, forming taste-selective pathways. B. The across fiber pattern theory. Taste quality is encoded in the activity of ensembles of neurons and the information is distributed among the ensembles. A neuron is activated by different taste qualities with different response magnitude (i.e., broadly tuned); a given taste is represented by the activated pattern or "landscape" of the neural ensembles. (Modified from (Lemon & Katz 2007), with permission under the terms of the Creative Commons Attribution License)

dominated in the central and ventral regions. However, acid responses were evenly distributed within the cortical taste area (Yamamoto 1984; Yamamoto et al. 1985a). These results suggest that the representation of taste in GC has a "relative chemotopic organization" (Yamamoto 1984; Yamamoto et al. 1985a). This finding was also observed using imaging techniques, which has the advantage of spatial resolution (Accolla et al. 2007; Yoshimura et al. 2004). Using *in vivo* intrinsic optical imaging, Accolla et al. found that different taste qualities activated specific spatial patterns containing both distinct and overlapping regions (Accolla et al. 2007). Although this technique does not provide single neuron resolution, their results suggest that those overlapping regions might contain a higher number of broadly tuned neurons, whereas regions responding to single taste quality might contain more narrowly tuned neurons. More recently, the development of *in vivo* calcium imaging with two-photon microscopy provides both spatial and single-neuron resolution (Stosiek et al. 2003; Dombeck et al. 2007; Sato et al. 2007; Kerr et al. 2007) and allows testing the "chemotopic map" hypothesis. However, two studies using this technique have reported conflicting findings. One study found sparse, "hotspot"-like representations of taste quality in superficial layers (Chen et al. 2011), while the other found both broadly and narrowly tuned neurons in mouse GC (Fletcher et al. 2015). Such conflicting evidence has left the debate on taste coding unsettled.

1.1.3. Beyond the Two Classical Theories

The debate on taste coding has monopolized the field over several decades, leaving many important phenomena unexplored. First, GC neurons respond to more than taste stimuli. Recordings in anesthetized rodents have shown that GC neurons responded to a variety of tactile stimuli in the oral cavity, such as "brushing the tongue" (Yamamoto 1984; Yamamoto, Yuyama, et al. 1984) and "probing the inside of the mouth with a glass rod" (Kosar et al. 1986). In addition, applying warm or cold water evoked different responses in GC (Kosar et al. 1986; Yamamoto 1984; Yamamoto, Yuyama, et al. 1984; Accolla et al. 2007). Thus, GC neurons also encode orosensory, i.e., tactile and thermal, properties of taste. Recent studies in freely licking rodents have also confirmed the responses of GC neurons to licking (Yamamoto et al. 1989; Yamamoto 1987; Katz et al. 2001; Stapleton et al. 2006). The mechanisms underlying the coding of those orosensory properties remain unexplored.

Second, GC encodes taste palatability. Palatability reflects the hedonic value or emotional valence of taste. It is a psychological dimension of the taste experience. For instance, sucrose is sweet and pleasant, while quinine is bitter and aversive. In animal studies, palatability is measured by the stereotyped orofacial behaviors evoked by infusion of different taste solutions. Typical orofacial behaviors are tongue protrusions for palatable taste and gaping for aversive solutions (Grill & Norgren 1978; Travers & Norgren 1986; Breslin et al. 1992; Spector et al. 1988). Palatability can also be assessed by measuring consumption, with palatable stimuli being consumed in larger volumes than aversive stimuli (Sadacca et al. 2012; Breslin et al. 1993; Smith et al. 1992). Behavioral studies, using lesions or inactivation, have shown that while the brain stem alone can produce palatability related motor patterns (Grill & Norgren 1978), the selection of an appropriate pattern is dependent on input from forebrain, including GC (Galaverna et al. 1992; Zardetto-Smith et al. 1994; Touzani et al. 1997; Luz et al. 2007; Fortis-

Santiago et al. 2010; Grill & Norgren 1978; Reilly et al. 2004; Reilly & Trifunovic 1999b; Touzani & Velley 1990; Touzani & Sclafani 2001; Reilly & Bornovalova 2005; Smith & Berridge 2005).

Two lines of research have investigated palatability coding in GC. One relies on correlation analyses. Recordings in GC have revealed that neural responses correlated with the taste palatability rank established by behavioral tests (Yamamoto et al. 1985b; Sadacca et al. 2012; Piette et al. 2012; Fontanini & Katz 2006). For instance, while citric acid (C) and quinine (Q) are unpalatable, sucrose (S) and NaCl (N) are palatable (at the concentration used in the cited research). In other words, the four tastants have the palatability relationship as: S > N >> C > Q. Neural responses in GC were found to correlate or anti-correlate with this relationship (Yamamoto et al. 1985b; Sadacca et al. 2012; Piette et al. 2012; Fontanini & Katz 2006). Activity evoked by C and Q were similar, while different from those by S and N, and vice versa. A recent study took advantage of NaCl, which dissociates taste intensity with palatability, to investigate palatability coding in GC (Sadacca et al. 2012). While the intensity of NaCl increases as increasing its concentration, the palatability curve against the increasing concentration of NaCl is an inverted "U", with medium concentration as the most palatable (Sadacca et al. 2012; Breslin et al. 1993; Smith et al. 1992). The authors found that overlapping groups of neurons in GC correlated with sodium concentration or the palatability rank (Sadacca et al. 2012).

The second line of research relied on a learning paradigm called conditioned taste aversion (CTA). In CTA learning, a sweet, palatable taste, e.g., saccharin, is turned into aversive by pairing it with gastric malaise. After just one pairing with intraperitoneal injection of LiCl, a salt inducing gastric distress, the sweet taste becomes unpalatable and rejected by the animal (Nachman & Ashe 1973; Bermúdez-Rattoni et al. 1986; Gallo et al. 1992; Grossman et al. 2008). Behavioral studies using lesions or pharmacological inactivation have shown that GC is important for CTA (Escobar et al. 1998; Gallo et al. 1992; Schier et al. 2014). By comparing neural responses of the same GC neurons to saccharin before and after CTA learning, Grossman et al. found that the palatability change of saccharin caused changes in GC neural responses (Grossman et al. 2008).

1.1.4. Taste Coding in GC is Temporally Dynamic

Recordings in anesthetized animals have provided a basic understanding of coding in GC: GC coding is multimodal, accounting for orosensory, chemosensory, and palatability information. However, previous studies only focused on how each single component was encoded and analyzed neural activity by averaging over 3-5 seconds (Yamamoto et al. 1985a; Yamamoto et al. 1989; Ogawa et al. 1990; Hanamori et al. 1998). An important question remained unanswered: could multiple dimensions of taste be encoded by GC neurons over time. This question is particularly important in regard to behavioral studies that have shown rodents can make a decision based on sampled tastants within several hundred milliseconds (Halpern & Tapper 1971; Graham et al. 2014; Perez et al. 2013).

In a seminal study, Katz et al. analyzed the time course of taste responses recorded in alert rodents and proposed a temporal dynamic model for taste processing in GC (Katz et al. 2001). In this study, the authors found that GC taste responses, evoked by passive deliveries via

intraoral cannula (IOC), were time-varying (i.e., firing rates evoked by taste change over time) and that different variables were encoded in different epochs following taste deliveries. The authors proposed that the time-varying firing rates of GC neurons could encode three variables in three distinct epochs: somatosensation, chemosensation, and palatability. The earliest epoch (0 – 200 ms) encodes the tactile sensation evoked by solutions striking the tongue, the second (starting after 200 ms) is devoted to coding the chemical properties of taste, and the third (starting after 1 s) encodes the palatability of taste (Figure 1.3) (Katz et al. 2001).

This work suggest that GC does more than coding taste quality; it also encodes orosensory, chemical and palatability information in time-varying patterns of spiking activity. The importance of this research has been in emphasizing that GC is multimodal and that taste coding is dynamic.

1.1.5. GC Encodes Taste Expectation

Taste is always expected. The sight of a can of Coca-Cola leads to an anticipation of its taste; the aroma coming from the kitchen will motivate you to expect a good supper. Recent research has revealed that GC can encode taste expectation. Two research lines were developed to study taste expectation in GC.

The first investigated the effects of expectation on taste responses. Similar to studies investigating attentional modulation on visual processing, which compared visual responses with or without attention (Wurtz & Mohler 1976; Motter 1993; Desimone & Duncan 1995; Luck et al. 1997; Ito & Gilbert 1999; Reynolds & Chelazzi 2004), studies on taste expectation compared taste-evoked neural responses with or without expectation. Several functional magnetic resonance imaging (fMRI) studies have shown that expectation of a specific taste can bias both perception and taste-evoked activity in GC (Nitschke et al. 2006; Veldhuizen et al. 2011). Nitschke et al. gave subjects different bitter taste stimuli ranging from mildly bitter to highly bitter while imaging brain activity using fMRI. The subjects, subsequently, rated the bitterness of received taste. Before receiving each gustatory stimulus, the subjects were cued with a verbal indication as to which bitter stimulus they could expect to receive. The cue could match or mismatch the upcoming taste. The authors found that the mismatched verbal cue, “mildly bitter”, reduced the perceived bitterness of the delivered highly bitter taste, and dampened the blood-oxygen-level dependent (BOLD) signal in GC (Nitschke et al. 2006). Later, a study with a similar task design showed that GC BLOD signals were biased toward sweet taste by a verbal cue, when a tasteless solution was given (Veldhuizen et al. 2011).

While these human fMRI experiments suggest that overall GC activity can be modulated by the expectation to a specific taste, studies in rodents have demonstrated that taste expectation can affect taste coding at single neuron and neural ensemble levels. Using a block trial design, Yoshida et al. compared taste-evoked activity between self-administered and passively delivered taste solutions (Yoshida & Katz 2011). In the self-administered block, rats were trained to self-initiate each trial and make a decision based on the taste sampled in a center port. This design ensured that rats would receive expected taste stimuli in the task. In a second block of trails,

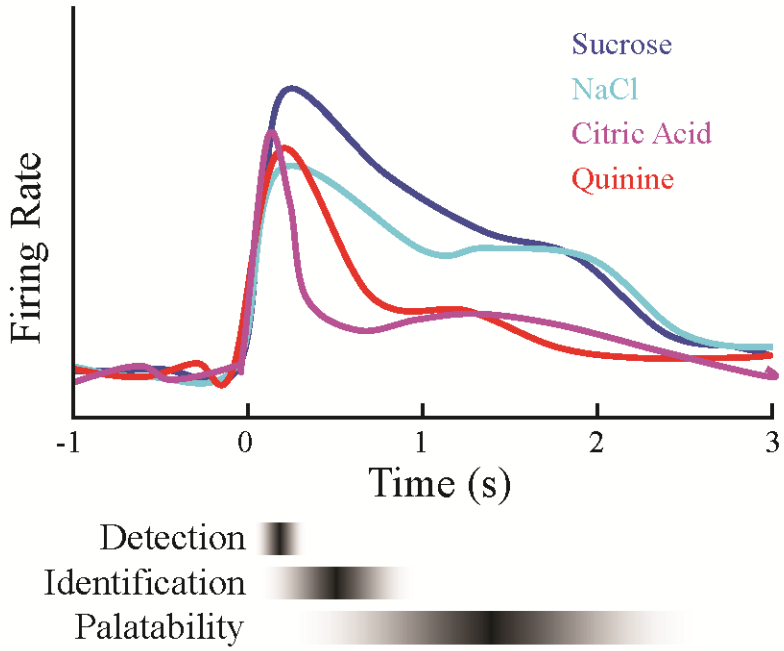


Figure 1.3: Katz model of taste coding. The upper panel shows a hypothetical neuron's responses to four basic taste (sucrose, NaCl, citric acid, quinine). Taste is deliveried at time zeros via intra-oral cannula. X axis: time, y axis: firing rate. The lower panel shows three epoches of taste coding. Initial responses are similar across tastants representing taste striking the tongue (the detection epoch). The identification epoch shows different firing rates across four tastants. The palatability epoch shows that firing rates are grouped according to the hedonic value of tastants. (Based on (Katz et al. 2001), with permission of The Journal of neuroscience)

passive taste deliveries were given without notice, and hence unexpected. This design allowed to parsing out the effects of expectation on taste responses. The taste expectation was in a general form, since taste solutions were randomly chosen from four basic tastants (sucrose [S], NaCl [N], citric acid [C], and quinine [Q]). The authors found that taste responses to the same tastants were different between the two conditions. Further analyses showed that the strongest response among the four tastants stayed the same, whereas other responses were suppressed in the self-administered condition compared with those in the passive condition. This result suggests that general expectation leads to a sharper tuning and improves the taste coding in GC (Yoshida & Katz 2011). In another study, Samuelsen et al. compared taste responses to self-administered taste deliveries after a cue with those to passively delivered taste stimuli during the inter-trial interval (ITI), to investigate the expectation modulation in GC (Samuelsen et al. 2012). The authors also observed an improvement in taste coding due to expectation. By analyzing the time course of taste coding, the authors demonstrated that the improvement of taste coding was due to the acceleration of taste coding induced by the taste expectation (Samuelsen et al. 2012). Both studies identified modulations of neural activity prior to taste deliveries, and this result suggests the effects of taste expectation should be in place before receiving taste.

The second research line on taste expectation focuses on responses evoked by taste-predictive contexts or stimuli. Early studies showed anticipatory modulation in GC neurons of freely ingesting rats (Yamamoto 1987; Yamamoto et al. 1989; Stapleton et al. 2006; Stapleton et al. 2007). In the 80s, Yamamoto et al. first observed that GC neurons could be activated right before licking taste solutions from a spout (REF: Yamamoto et. al. 1987). However, the authors did not rule out the possibility that the modulation could be driven by preparatory, oromotor related activity. Recently, Stapleton et al. used a fixed ratio schedule (FR5) paradigm to show that GC neurons displayed anticipatory activation on top of licking related activity (Stapleton et al. 2006). In this study, rats were required to initiate a sequence of licks and received a drop of random chosen taste solution upon the fifth lick. The authors found that GC neurons fired differently between the second and the fourth licks. Since all licking behaviors share the same motor action, the result suggests the coding of taste anticipation in GC prior to receiving taste (Stapleton et al. 2006). In a follow-up study, the authors showed that when the same tastants were delivered within blocks, the anticipatory activities during dry licks differed across blocks. It suggests that anticipatory activity in GC can contain information about the identity of upcoming taste (Stapleton et al. 2007).

While important in suggesting the existence of anticipatory activity in GC, these studies did not address how anticipatory signals are encoded by GC. First, taste expectation was implicit in these tasks and it was not clear when rats started to expect taste. Second, anticipatory activities shown in these studies were accompanied by the concurrent licking, which could confound the interpretation of taste expectation. Third, the block design, although separated the taste quality, introduced potential confound factors such as internal state modulations (i.e., satiety due to the time passing). These issues can be resolved by using the cue-outcome pairing procedure that has been widely used in studying reward association and outcome expectancy in limbic and higher-order regions (Schoenbaum et al. 1998; Schoenbaum et al. 2003; Schultz 1997; Cohen et al. 2012). This procedure uses a salient sensory stimulus, e.g., tone or odor, as the cue to predict the receiving of taste. Upon forming the association between cue and taste, the onset of the cue

signals the expectation of taste. Thus, a well-defined temporal trigger can be used to investigate the coding of taste expectation.

While studies using fMRI in human have suggested the cue-induced activation in the taste cortex (Tang et al. 2012; Van der Laan et al. 2011), evidence demonstrating cue responses at single-neuron level in GC first comes from studies using immediate early genes (IEGs) (Kerfoot et al. 2007; Saddoris et al. 2009). IEGs, for example c-fos, transcribe about 30 minutes to an hour following periods of high neuronal activation. Thus, their products can be revealed *post hoc* to mark neurons activated by a relevant behavior or stimulus in the experiment. Using c-fos labeling, Kerfoot et al. first reported that a sucrose predicting tone could activate neurons in GC (Kerfoot et al. 2007). In a later study, Saddoris et al. advanced our understanding on the relationship between cue- and taste- activated neurons, by taking advantage of the different temporal-spatial transcription pattern between two IEGs (i.e., Arc and Homer1a). In this study, the authors used fluorescent in situ hybridization (FISH) to label neurons activated by sucrose itself and those by a sucrose-predicting odor in the same brain section. Examining the double-labeled neurons, the authors showed that a group of neurons in GC were activated by both cue and sucrose (Saddoris et al. 2009). These results have demonstrated that GC neurons can be activated by the taste predictive cue and most of them are the neurons that encode the predicted taste as well. The above studies suggest that taste predictive cues evoke taste expectation by activating neurons process taste information.

More recent studies combined *in vivo* electrophysiology and the cue-taste pairing behavioral procedure to directly address the coding of taste expectation in GC. Samuelsen et al. investigated, for the first time, how anticipatory cues are encoded in GC (Samuelsen et al. 2012). The authors trained head-fixed rats to expect taste delivery by responding to an auditory cue with a lever press, while recording neural activity in GC. They found that a group of neurons in GC showed excitatory or inhibitory modulations when the cue was played (Figure 1.4A). The same auditory stimulus triggered minimum modulations in GC neurons recorded in un-trained animals, hence cue responses were mediated by learning. Analyzing neural activity triggered by lever presses in the inter-trial interval showed little modulation. Comparing the timing of cue response onset with orofacial movements showed that cue responses happened earlier than the detectable mouth movements (Samuelsen et al. 2012). These results demonstrated that GC encodes taste expectation by responding to the taste cue. In another study, Samuelsen et al. showed that expectation also induced similar cue responses in a classical conditioning procedure, where the auditory cue preceded the subsequent passive taste deliveries (Samuelsen et al. 2013).

Gardner and Fontanini (Gardner & Fontanini 2014) further investigated the roles of cue responses in GC. To address if specific taste cues would evoke different cue responses, Gardner and Fontanini used a Go/No-Go task in a head-fixed preparation combined with electrophysiological recordings in GC (Gardner & Fontanini 2014). In this study, the authors trained rats to associate one cue with the availability of sucrose and another cue with the availability of quinine. Rats learned to press a lever following the sucrose-anticipating cue (and self-administer sucrose) and to refrain from pressing for the quinine-anticipating cue. They found that GC neurons did show different responses to the two cues. This cue-selectivity emerged with learning and the sucrose cue responses could be reduced by extinction procedures. More importantly, the sucrose cue evoked activity resembled the activity evoked by sucrose itself. By

introducing omission trials, the authors found, even without sucrose, sucrose cue can lead to sucrose-like responses in a group of neurons (Gardner & Fontanini 2014). Therefore, GC can encode specific taste expectation by specific cue responses.

1.1.6. Expectation Evoked Activity in GC Drives Food Seeking Behaviors

Previous studies in reward related circuits have shown that the expectation encoded by cue responses is important for motivating behaviors such as food and reward seeking (Berridge 2012; Cardinal et al. 2002; Petrovich 2011). The evidence that GC can encode taste expectation and respond to cues suggests the cue related activity in GC may be essential for food seeking. Part of my dissertation study was devoted to test this hypothesis. We collaborated with Dr. Kusumoto-Yoshida, Dr. Chen, and Dr. Bonci at the National Institute of Drug Addiction (NIDA) and conducted a study to test the role of anticipatory activity in GC (Kusumoto-Yoshida et al. 2015). The details of this study will be presented in Chapter 4. GC is a major portion of the insular cortex (IC); and in this study IC and GC were used interchangeably. Briefly, we developed a Pavlovian conditioning task, where mice learned an 11 s compound cue (tone + light) would lead to a food pellet delivery in a food port at the 10^{th} second. After learning, mice showed an increase of port entries during the cue. We found neurons in mouse GC displayed significant modulation during cue presentation, as well as during food consumption. Inactivating GC activity by drug infusion or inhibiting GC activity only during the cue by optogenetics impaired the conditioned port checking behavior. These results demonstrated that the cue-induced activity in mouse GC was necessary for the expression of the cue-induced behavioral responses in the Pavlovian conditioning. Thus, cue activity in GC, like cue signals in reward circuits, has significant behavioral consequences.

1.2. Mechanisms Underlying the Integration of Sensory and Affective Signals in GC

How can GC, a primary sensory cortex, show such a complex and rich array of functions and encode different physiochemical and psychological variables? Classically, the integrative function of GC is believed to emerge through a convergence of anatomical inputs carrying sensory and limbic information (Katz et al. 2002; Maffei et al. 2012).

1.2.1. BLA Inputs Provide the Affective Dimension of Taste Experience in GC

Limbic inputs to GC come from multiple sources, such as mediodorsal thalamus, BLA, LH, VTA, and parahippocampal region (Maffei et al. 2012; C. Shi & Cassell 1998; Saper 1982; Ohara et al. 2003; Agster & Burwell 2009). Among all the limbic inputs, the one from BLA is the most studied. BLA has gained a lot of attention for its close relationship with taste processing and its roles in emotion and reward associations. First, anatomically, taste related information arrives in BLA via ascending fibers from PBN in the brainstem (Norgren 1976; Veening 1978; Bernard et al. 1993) and projections from GC (Yamamoto et al. 1984; Bielavska & Roldan 1996). BLA is also known to receive inputs from other regions processing reward and value such

as VTA and OFC (Cassell & Wright 1986; Albanese & Minciacchi 1983). Second, BLA neurons have been shown to respond to taste administrations and process the hedonic valve of taste (Yasoshima et al. 1995; Scott et al. 1993; Nishijo & Uwano 1998; Grossman et al. 2008; Fontanini et al. 2009). Reward predicting cues also activate BLA neurons (Fontanini et al. 2009; Schoenbaum et al. 1998; Roesch et al. 2010). Third, BLA lesions not only impair the learning of reward association and CTA (Yamamoto 1993; Bermúdez-Rattoni et al. 1986; Gallo et al. 1992; Nachman & Ashe 1973; Quirk et al. 1995; Rollins et al. 2001), but also alter taste-related behaviors (Touzani et al. 1997; Ganaraja & Jeganathan 2000). Moreover, bidirectional manipulations of the BLA-GC pathway modulate CTA learning, which involves the change of taste palatability. Enhancing BLA-GC projections by drug infusion (Ferreira et al. 2005) or electrical stimulation (Escobar & Bermúdez-Rattoni 2000) enhances CTA learning; while blocking the long-term potentiation (LTP) of the BLA-GC projection impairs CTA (Escobar et al. 1998).

Several recording studies have been conducted to examine the contribution of BLA-GC projections to the taste coding in GC. BLA contributes to palatability coding in GC. Recent studies, through time course analyses of neural responses, have shown that palatability information appears earlier in BLA than in GC, suggesting a transfer of palatability information from BLA to GC (Katz et al. 2001; Fontanini et al. 2009). Recordings simultaneously from BLA and GC in rats undergoing CTA learning also support this hypothesis (Grossman et al. 2008). Taking advantage of CTA learning, Grossman et al. found that neural responses to saccharin, both in BLA and GC neurons, were changed before and after learning. While changes in BLA were observed throughout the analysis period (both early and late), most changes in GC were restricted to the late, "palatability-coding" epoch (1-3 sec after taste delivery). By analyzing the cross correlation between the spike trains of BLA and GC neurons recorded simultaneously, the authors found CTA learning enhanced the functional connectivity between BLA and GC (Grossman et al. 2008). These results suggest that increased BLA-GC communication mediates CTA learning and supports the BLA-to-GC transferring of palatability information. More recently, Piette et al. directly tested the hypothesis that BLA is responsible for GC palatability coding, by recording GC taste responses when inactivating BLA pharmacologically (Piette et al. 2012). The authors found that BLA inactivation led to changes of GC neuronal responses to taste, which happened mostly in the late epoch (1-3 sec after taste delivery). Through detailed analyses, they demonstrated that only palatability coding was abolished by BLA inactivation (Piette et al. 2012).

BLA also contributes to the coding of taste expectation in GC. Previous studies have shown that BLA neurons respond to taste predicting cues (Fontanini et al. 2009). Samuelsen et al. found that the cue responses in BLA happened earlier than those in GC. Moreover, BLA pharmacological inactivation resulted in significant reduction of the cue responses in GC (Samuelsen et al. 2012). These results suggest that BLA is responsible for the cue responses in GC.

Altogether, these results demonstrate that BLA inputs provide the affective dimension of the taste experience for GC, including palatability and taste expectation.

1.2.2. VPMpc Provides the Sensory Dimension of Taste Experience in GC

While the function of the BLA-GC connection has been extensively studied, our knowledge of the role of VPMpc in carrying sensory information is limited. There is only one study, which investigated the thalamocortical contribution to taste coding in GC of alert animals (Samuelsen et al. 2013). In this study, Samuelsen et al. recorded GC activity while inactivating VPMpc pharmacologically (Samuelsen et al. 2013). The authors found that VPMpc inactivation changed taste responses for more than 75% of GC neurons and dramatically reduced the ability of GC neurons to encode taste quality. In the second experiment, the authors conditioned taste deliveries by playing an auditory cue 1 s before them. Similarly to previous studies (Samuelsen et al. 2012), the cue in classical conditioning also activated GC neurons. Those cue responses, as measured by averaging across neurons, however, survived thalamic inactivation. This result suggests that VPMpc is a major contributor to taste responsiveness in GC, and that its role in shaping cue responsiveness might be negligible (this suggestion is now known to be only partially correct – see below).

Altogether, these results seemed to be consistent with a classical dichotomic model of taste processing (Yamamoto 2006; Maffei et al. 2012) (Figure 1.4B). This view postulates the existence of two distinct channels of information, a thalamic channel and a limbic channel, one conveying chemosensory signals and the other conveying limbic anticipatory and reward related signals. While appealing in its elegance and simplicity, this view is at odds with some data from the literature on the VPMpc.

1.3. Functional Roles of the Gustatory Thalamus

Historically, the gustatory thalamus has been viewed as a passive relay for sensory information (Yamamoto 2006; Maffei et al. 2012; Carleton et al. 2010). Previous electrophysiological studies in VPMpc focused on the encoding of basic orosensory and chemosensory properties of taste in anesthetized rodents. By averaging stimulus-evoked neural responses in VPMpc over several seconds, previous studies have shown that VPMpc neurons respond to tactile stimuli on the tongue and neural responses differ across different temperature and taste quality (Erickson et al. 1965; Emmers 1966; Emmers 1966; Scott & Erickson 1971; Ganchrow & Erickson 1972; Scott & Yalowitz 1978; Nomura & Ogawa 1985; Ogawa et al. 1987; Ogawa & Nomura 1988; Verhagen et al. 2003). Some studies also investigated the receptive fields of taste and mechanosensitive neurons in VPMpc (Ogawa & Nomura 1988). The most recent study (Verhagen et al. 2003) has shown that more than 40% of VPMpc neurons were multimodal, i.e., encoding a combination of tactile, thermal and chemosensory information. Taste quality encoding neurons were broadly tuned, as they responded to multiple tastants (Verhagen et al. 2003). In addition, time-varying firing activity has been observed and analyzed by averaging responses across the whole population (Verhagen et al. 2003). In a follow-up study, the authors applied an artificial neural network to analyze the taste coding in thalamic taste responses and found that considering the temporal information increased the accuracy of taste discrimination by the artificial network (Verhagen & Scott 2004).

While all these data are consistent with a view of the VPMpc as a relay of chemosensory and orosensory information, other evidence points at a more complex role for this nucleus. Single unit recordings in the VPMpc of anesthetized rats suggest that neurons may encode palatability (Scott & Erickson 1971; Verhagen et al. 2003). To visualize taste quality representations, Verhagen et al. projected population neural responses of each taste stimulus into a 3-dimension space based on the similarity of population activity across all taste stimuli. Tastants belonging to the same taste categories clustered together and separated from different categories. For example, all sodium salts were close to each other but far from sucrose-fructose tastants. Interestingly, the authors noted that there was a trend on the z axis, with sucrose and low concentration sodium solutions occupying the lower part and acid and bitter taste the higher. This result suggests a "hedonic tone" and implies the coding of taste palatability in VPMpc (Verhagen et al. 2003).

Evidence from recording and behavioral studies have suggested that VPMpc might also participate in processing taste expectation. Due to the relative small size, the function of VPMpc in basic taste behavior, such as taste detection, had been controversial (Oakley & Pfaffmann 1962; Grill & Norgren 1978; Lasiter 1985; Lasiter et al. 1985; Flynn et al. 1991; Flynn et al. 1991; Reilly & Pritchard 1995; Reilly & Pritchard 1996b; Reilly & Pritchard 1996a; Scalera et al. 1997; Reilly 1998). Recent lesion studies have shown that VPMpc lesions do not disrupt basic taste functions such as taste detection and discrimination (Reilly & Pritchard 1996b; Reilly & Pritchard 1996a; Scalera et al. 1997; Reilly 1998). However, VPMpc lesions impair complex, taste guided behaviors involving higher-order functions (Reilly 1998; Reilly & Trifunovic 2003; Reilly et al. 2004; Schroy et al. 2005; Arthurs & Reilly 2013). For instance, after associating a palatable saccharin solution with the subsequent, more preferred sucrose solution, rats will reduce the intake of the saccharin in anticipation of the subsequent sucrose. This phenomenon is called anticipatory negative contrast (ANC) and is found to be impaired by VPMpc lesions (Reilly et al. 2004; Reilly & Pritchard 1996b; Schroy et al. 2005). It suggests that VPMpc might be important for and participate in anticipation.

While previous electrophysiology studies have not directly investigated the role of VPMpc in taste anticipation, some observations have suggested that VPMpc might process taste anticipation. Pritchard et al. recorded and analyzed taste response in VPMpc in alert macaque monkeys (Pritchard et al. 1989). The authors observed that a group of VPMpc neurons modulated their firing activity prior to water rinse, which was conducted in a fixed schedule after every taste delivery. This led the authors to speculate the potential function of VPMpc in anticipatory behaviors. However, as noted by the authors, this anticipation-like activity was confounded by the concurrent preparatory oro-motor behavior (Pritchard et al. 1989).

Another hint on the involvement of VPMpc in processing taste anticipation came from recent results from our lab (Figure 1.5). Although VPMpc inactivation did not change the overall cue responsiveness of GC (Samuelsen et al. 2013), new results from our lab showed that the temporal dynamics of individual cue responses were dramatically changed by inactivating the thalamus (Figure 1.5; Samuelsen et al. unpublished data). Figure 1.5A shows two representative examples. Both of them display significant cue responses before and after VPMpc inactivation. However, the response temporal profiles are dramatically changed. The upper one changes from a long-lasting response to a phasic one; the lower one is dramatically reduced after VPMpc

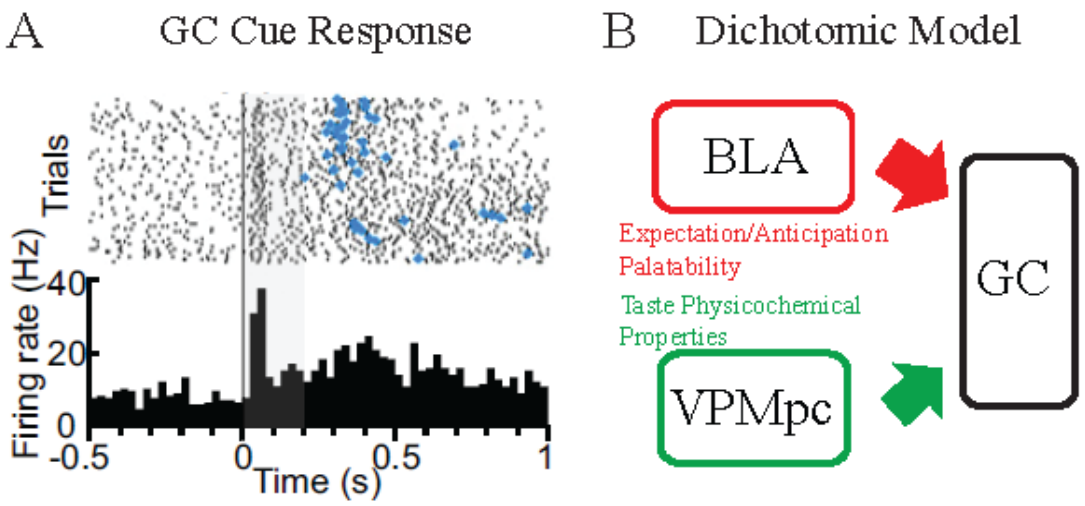


Figure 1.4: Cue responses in GC and the dichotomic model for the integration of information in GC. A. A representitive cue responsive neuron in GC. Upper: raster plot, blue dots are level presses. Lower: peri-stimulus time histogram (PSTH). X axis is time, cue played at time zero; y axis is firing rate. The cue evoked a phasic response before any lever press and taste response. (Modified from (Samuelsen et al. 2012), with permission of the Elsevier). B. A schematic representation of the dichotomic model for information processing in GC. Both BLA and VPMpc directly project to GC. According to the model, the former provides afffective and cognitive information (red), i.e., expectation and palatability; the latter provides pure sensory information (green), such as taste chemical identity.

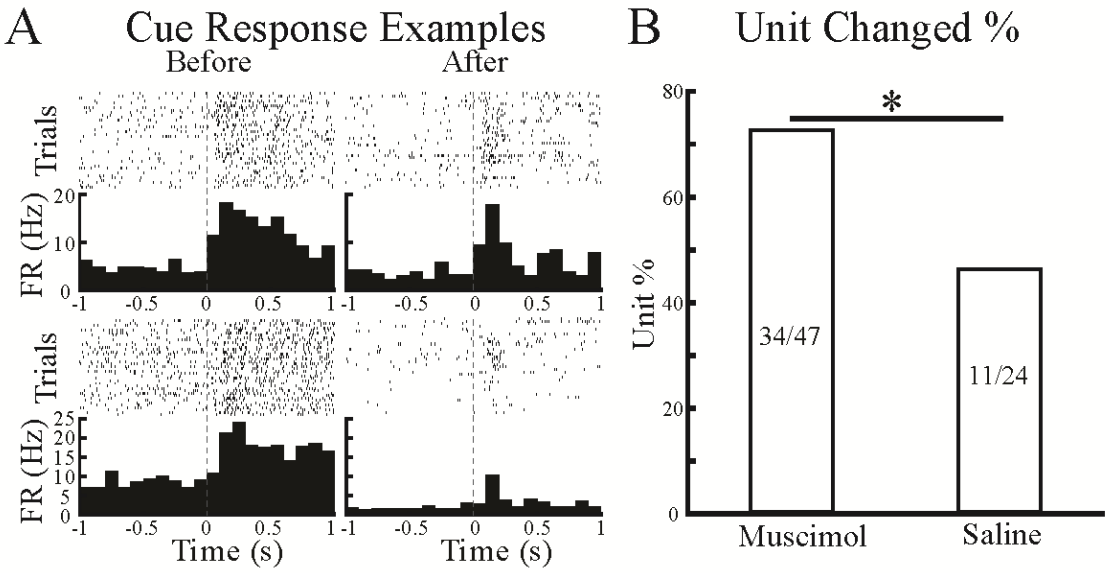


Figure 1.5: VPMpc inactivation changes the temporal dynamics of cue responses in GC. A. Raster plots and PSTHs of two representative neurons responding to the cue before and after muscimol infusion in VPMpc. They show different types of changes in the temporal dynamics of cue responses. B. Percentages of unit that show significant temporal dynamic changes in cue responses. The percentage changed in inactivation group (Muscimol) is significantly higher than that in the control group (Saline) (two proportion test, $z = 2.19$, $p = 0.029$).

inactivation. Analyzing the temporal pattern of cue responses (two-way ANOVA test [condition X time], $p < 0.05$), the authors found a significantly higher percentage of cue responsive neurons was affected by VPMpc inactivation comparing with that by saline control (two proportion test, $z = 2.19$, $p = 0.029$) (Figure 1.5B). This result suggests that VPMpc may play important roles in modulating cue responses. It implies that VPMpc may show cue related activity and be important for processing taste expectation.

In summary, previous studies have provided a basic understanding of how the VPMpc processes chemosensory and orosensory information in anesthetized animals. This seems to be consistent with the dichotomic model of GC integration, where the thalamocortical pathway provides only sensory information and the amygdalar-cortcial pathway provides the psychological dimension. However, little is known on how this nucleus encodes sensory information in alert animals. In addition, evidences from both recording and lesion studies suggest the involvement of VPMpc in coding psychological dimensions of the taste experience, such as taste palatability and taste anticipation. In this dissertation, we have conducted a series of experiments to investigate taste coding in the VPMpc of alert rats, specifically addressing its ability to encode expectation. The findings not only provide important updates on information processing in VPMpc of alert animals, but also present information that challenges the dichotomic model of taste processing.

1.4. Experimental Design and Dissertation Structure

In this dissertation, we have designed and conducted experiments to investigate the information processing in VPMpc of alert rats. The experiments focus on understanding: 1) taste coding in VPMpc of alert rats; 2) the effects of general expectation on taste coding in VPMpc; 3) the coding of anticipatory cues in VPMpc.

Our experiments are based on single unit recordings in alert rats engaged in a behavioral task. Rats were implanted with movable bundles of electrodes in VPMpc and intra-oral cannulae (IOCs) in the mouth for taste delivery. The animals were trained on a cued-self-administration task. In this task, rats learn to self-deliver a taste solution by entering a nose port after a brief auditory cue. The taste delivered at each trial was chosen randomly from basic taste solutions (S, N, C, and Q). The cue did not provide information on the identity of each tastants, but was a general "go" signal. This design allowed us to study expectation of taste in its most general form. During the inter-trial interval (ITI), rats also received passive taste deliveries without any notice. Results of these experiments are presented in the following chapters.

In Chapter 2, I present the results on the temporal dynamics of taste responses and taste coding in VPMpc of alert rats. Our analyses describe the temporally dynamic nature of taste processing in VPMpc. We also show evidence that VPMpc neurons process taste palatability information. By comparing responses to passively delivered tastants with those evoked by self-administrations, we investigate the effects of taste expectation on taste processing in VPMpc.

In Chapter 3, I focus on the activity evoked by the taste-predicting cue. Neurons in VPMpc are found to respond to the taste predicting cue. Additional experiments have been conducted to demonstrate that cue responses are not driven by other factors such as mouth movements, but encode taste anticipation. Analyzing the cue response magnitude in relation to the animal's behavior show that cue responses are state-dependent and the strength of cue responses predicts the animals' behavioral response.

In Chapter 4, I present a study on the behavioral significance of cue signals in the taste system. This study is the result of a collaboration between our laboratory and the group led by Dr. Antonello Bonci at NIDA. We combine behavioral electrophysiology with pharmacological and optogenetic manipulation of GC to investigate the functional role of cue-related activity. A Pavlovian conditioning procedure is used to assess conditioned behavioral responses upon food cue. Electrophysiological recordings show the cue induces strong modulations on neural activity in GC. The suppression of cue-induced, conditioned behavioral responses by both drug infusion and optogenetic inactivation reveals the important role of cue signals in GC for mediating food seeking behavior.

In Chapter 5, I discuss our findings in relation with taste coding in GC and speculate the behavioral significance and the coding of cue responses in the taste system. Chapter 6 is devoted to the materials and methods, as well as analysis detail.

Chapter 2

State Dependency of Chemosensory Coding in the Gustatory Thalamus of Alert Rats

2.1. Introduction

The function of the gustatory thalamus remains largely unexplored. Neurons in the parvicellular portion of the ventroposteromedial nucleus of the thalamus (VPMpc) receive inputs conveying gustatory information from the parabrachial nucleus (PbN) (Ogawa et al. 1987; Karimnamazi & Travers 1998; Bester et al. 1999; Krout & Loewy 2000; Paxinos 2004; Holtz et al. 2014). VPMpc neurons process ascending gustatory signals and send their output to the gustatory cortex (GC) (Pritchard et al. 1989; Allen et al. 1991a; C. J. Shi & Cassell 1998; Nakashima et al. 2000; Verhagen et al. 2003; Samuelsen et al. 2012). The VPMpc exerts a strong influence on GC ongoing and evoked activity. Pharmacological silencing of VPMpc changes the state of gustatory cortical networks resulting in slow and synchronous activity. In addition, inactivation of the VPMpc dramatically alters taste-evoked dynamics and greatly reduces neurons' ability to encode taste in GC (Samuelsen et al. 2013). The function of the VPMpc is not limited to the processing of physiochemical signals (Reilly 1998). Behavioral experiments have revealed that lesions of VPMpc lead to deficits in anticipatory contrast and autoshaping (Reilly & Pritchard 1996a; Reilly & Pritchard 1997), suggesting an involvement of the thalamus in the anticipation of gustatory rewards.

Despite the key role of the VPMpc for cortical processing of gustatory information, little is known on how thalamic neurons encode taste. Most of our knowledge about the VPMpc of rodents comes from experiments in anesthetized or paralyzed animals (Scott & Erickson 1971; Scott & Yalowitz 1978; Nomura & Ogawa 1985; Ogawa & Nomura 1988; Verhagen et al. 2003). Recordings of single unit activity in urethane-anesthetized rats revealed that almost half of the VPMpc neurons respond to taste, and that taste responses are multimodal and broadly tuned. In addition, these experiments suggested that the VPMpc can encode the hedonic value of taste (Verhagen et al. 2003). While these pioneering studies are fundamental in shaping our basic understanding of thalamic processing, it is not clear how the patterns of activity observed in anesthetized rats relate to those recently described in other gustatory nuclei and areas of alert animals. Recordings in the PbN (Di Lorenzo et al. 2009; Rosen et al. 2011; Weiss et al. 2014) and in GC (Katz et al. 2001; Fontanini & Katz 2006; Jones et al. 2007; Samuelsen et al. 2013) showed that, in behaving rodents, neurons responded to taste with time-varying modulations of firing rates. Given the interconnectivity of the VPMpc with these two areas, it is likely that in alert animals thalamic neurons may also display heterogeneous temporal dynamics and multiplex gustatory information via time-varying modulations of firing rates. In addition, recent analyses of

taste coding in GC showed that temporal coding of gustatory information was shaped by the state of the animal. Specifically, expectation enhances taste coding by shortening the latency at which gustatory information was encoded by GC spiking activity (Samuelsen et al. 2012). These results, together with data from other sensory systems (Fanselow & Nicolelis 1999; Krupa et al. 1999; Nicolelis & Fanselow 2002a; Cano et al. 2006; Lesica et al. 2006; McAlonan et al. 2008; Saalmann & Kastner 2011), suggest that also VPMpc might modify its temporal coding scheme according to the anticipatory state of the animal.

Here we present results from single unit recordings in alert rats unveiling how the VPMpc processes chemosensory information. Single units were recorded in response to intraoral infusions of tasting solutions. First, we analyzed taste coding for passively delivered tastants, focusing on the temporal dynamics of chemosensory and palatability coding. The data revealed rich temporal dynamics in single neuron responses with fast onset of taste processing. We then compared, for each neuron, responses to passively delivered tastants with responses to cued and self-delivered stimuli. This paradigm was used to probe the effects of general expectation on taste processing. We found that the general anticipatory state of the animal significantly influences the response profile of single neurons.

To our knowledge, our results provide the first description that taste coding in VPMpc is dynamic and state dependent, and that expectation improves and accelerates the thalamic coding of taste.

2.2. Results

128 single units (Figure 2.1A) were recorded from the VPMpc of 11 rats engaged in a behavioral paradigm involving passive deliveries and self-administrations of tasting solutions (Figure 1B). Electrode positioning was verified with *post-hoc* histological reconstruction of recording tracts (Figure 2.1C). Only recordings from electrodes passing through the VPMpc were used for analysis.

2.2.1. VPMpc Neurons Respond to Taste with Time-varying Modulations in Firing Rates

To assess how taste affects spiking activity of VPMpc neurons, we analyzed the time course of firing rate modulations in response to tasting solutions passively delivered into the mouth via an intra-oral cannula. Figure 2.2A shows raster plots and peristimulus time histograms (PSTHs) for two representative neurons, one excited (top) and one suppressed (bottom) by taste delivery. Use of a change point analysis (Jezzini et al. 2013) (see 6.2.2) revealed that 92 out of 128 units (71.9%) showed spiking dynamics that were significantly modulated (either enhanced or depressed) by taste delivery. These neurons were defined as taste-responsive (TasteR). In total, taste delivery suppressed 37 neurons, excited 34 neurons and had mixed effects on 21 neurons. When individual responses to tastants were analyzed (total response number = 4 × 128), 196 were significantly different from baseline: 108 were excitatory and 88 inhibitory. Figure 2.2

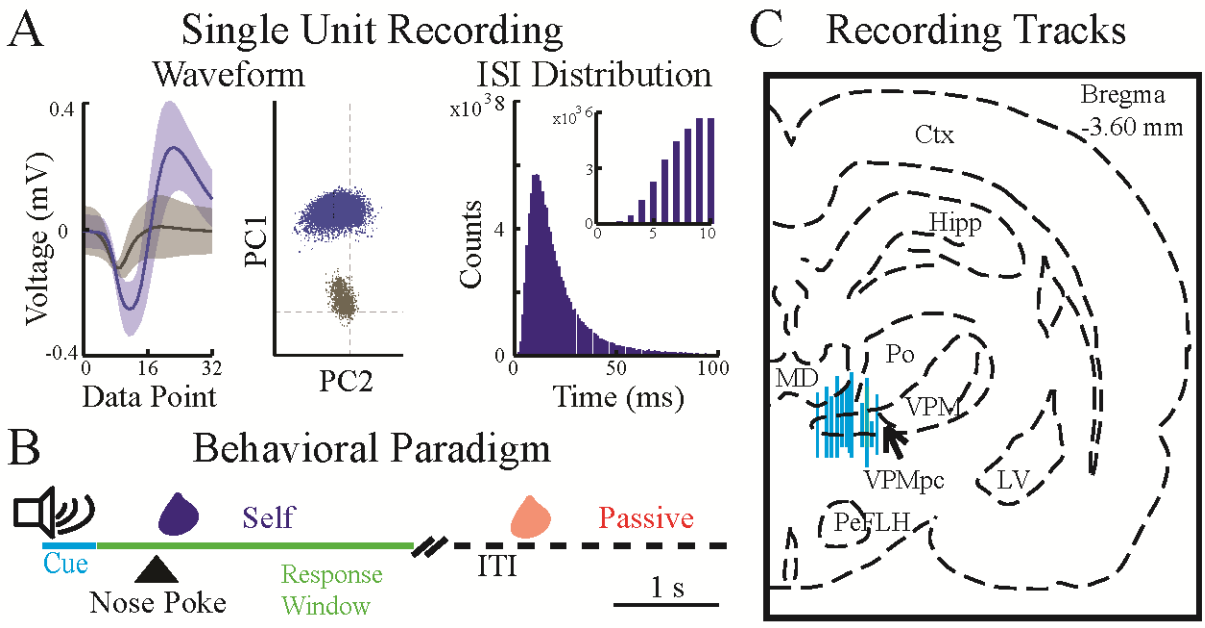


Figure 2.1: Experimental procedures. A. Representative single unit recording in VPMpc. Left: average single unit spike waveform (blue) and noise (grey); shadings indicate 95% confidence interval. Middle: principal component analysis of waveform shape for spikes (blue) and noise (grey). Dashed lines indicate 0. Right: inter-spike interval (ISI) distribution of action potential for the isolated waveform. Insert: zoom-in from 0 to 10 ms. B. Schematic representation of the behavioral paradigm for passive deliveries and self-deliveries. Each trial begins with a 0.5 s long auditory cue. Entering a nose port within 3 s from the offset of the cue triggers a self-administration (Self) of a taste solution. Taste solutions can also be delivered passively (Passive) during the inter-trial interval (ITI, from 30 to 50 s). Each taste delivery is followed by a water rinse (not shown). C. Schematic representation of a coronal section of the rat brain (modified from (Paxinos & Waston 2006), with permission of the Elsevier) showing the dorso-ventral range of recordings in gustatory thalamus (VPMpc). Blue lines indicate reconstructed recording tracks from the first to the last recording session. MD, mediodorsal thalamic nuclei; Po, posterior thalamic nuclear group; VPM, ventroposteromedial thalamic nuclei; VPMpc, ventroposteromedial thalamic nuclei, parvicellular; Hipp, hipocampus; LV, lateral ventricle; Ctx, cortex; PeFLH, perifornical part of lateral hypothalamus, orbital frontal cortex are not shown here. Feedback projections are not shown neither.

B shows population averages (i.e. PopPSTH) of excitatory and inhibitory responses. On average, excitatory responses showed significantly faster onset compared to inhibitory responses (227 ± 33 ms, N = 108, vs. 361 ± 41 ms, N = 88). This was confirmed by comparing response latency distributions of excitatory and inhibitory responses (Kolmogorov-Smirnov test, $p < 0.05$, see Figure 2.2B inset).

Neurons could show multiple firing rate modulations within the first 2.5 seconds (s) following taste delivery. Analysis of the distribution of modulation onset revealed that 69.2% (193 out of 279 modulations detected in 196 responses) of modulations occurred within the first 0.5 s, 16.5% (46/279) between 0.5 and 1 s and 14.3% (40/279) after 1 s (Figure 2.2C, top panel). The distribution of the duration of significant firing rate modulations was also analyzed (Figure 2.2C, bottom panel), revealing that while the median was 701.7 ms, the distribution was rather heterogeneous and spread over the entire 2.5 s (95% confidence interval: [29.4, 2332.2]). This result suggests a richness and variety of temporal profiles in VPMpc firing responses. Figure 2.2D, which shows the entire population of significant taste responses, illustrates the different time courses. To better extract the most frequent trends in the time course of responses, we applied a principal component analysis (PCA) on responses pattern (Narayanan and Laubach, 2009). Activity of significant responses (108 excitatory and 88 inhibitory) from -1 to 2.5 s around taste delivery was normalized using an auROC procedure (see 6.2.1). The first three principal components (PCs) accounted for 85.3% of total variance (PC1 64.9%, PC2 13%, and PC3 7.5%; Figure 2.2D, right). The Eigenvector that accounted for the largest variance represented a monotonic component (PC1) that outlasted the 2.5 s period examined. Additional response patterns involved mixed, either biphasic (PC2) or triphasic (PC3), modulations.

2.2.2. Processing of Taste Identity in VPMpc

After characterizing the temporal dynamics of taste-evoked responses, a series of analyses were performed to determine how information pertaining to taste identity is encoded by VPMpc neurons. Each of the taste-responsive neurons was analyzed using a two-way ANOVA (time X taste) to identify whether it responded differently to the four tastants (Jezzini et al. 2013; Samuelsen et al. 2013). Neurons that were taste-responsive and showed significantly different responses to the 4 tastants according to the two-way ANOVA were defined as taste-specific. This method yielded 56 taste-specific units from 92 taste-responsive units, which accounted for 43.7% of all recorded units (56/128). Taste-specific units were analyzed with a Euclidean distance-based classification algorithm (Jezzini et al. 2013) (see 6.2.4) to evaluate how many and which gustatory stimuli were encoded by each unit. Figure 2.3A shows a representative thalamic neuron whose firing is taste-specific. This neuron responded the strongest to citric acid, however it did not encode exclusively for citric acid. The time course of this neuron's response was different for each of the tastants, allowing for an above chance classification performance of all four tastants (Figure 2.3B, best two classifications for NaCl and citric acid). The coding profile of each VPMpc taste-specific neuron was analyzed. Similar percentages of neurons encoded for one (25%, 14/56), two (26.8%, 15/56), three (25%, 14/56) or four (23.2%, 13/56) taste qualities, as revealed by the distribution plot in Figure 2.3C. The average decoding performance of each taste-specific neuron was 0.43 ± 0.02 (N = 56). Performing the classification analysis using data

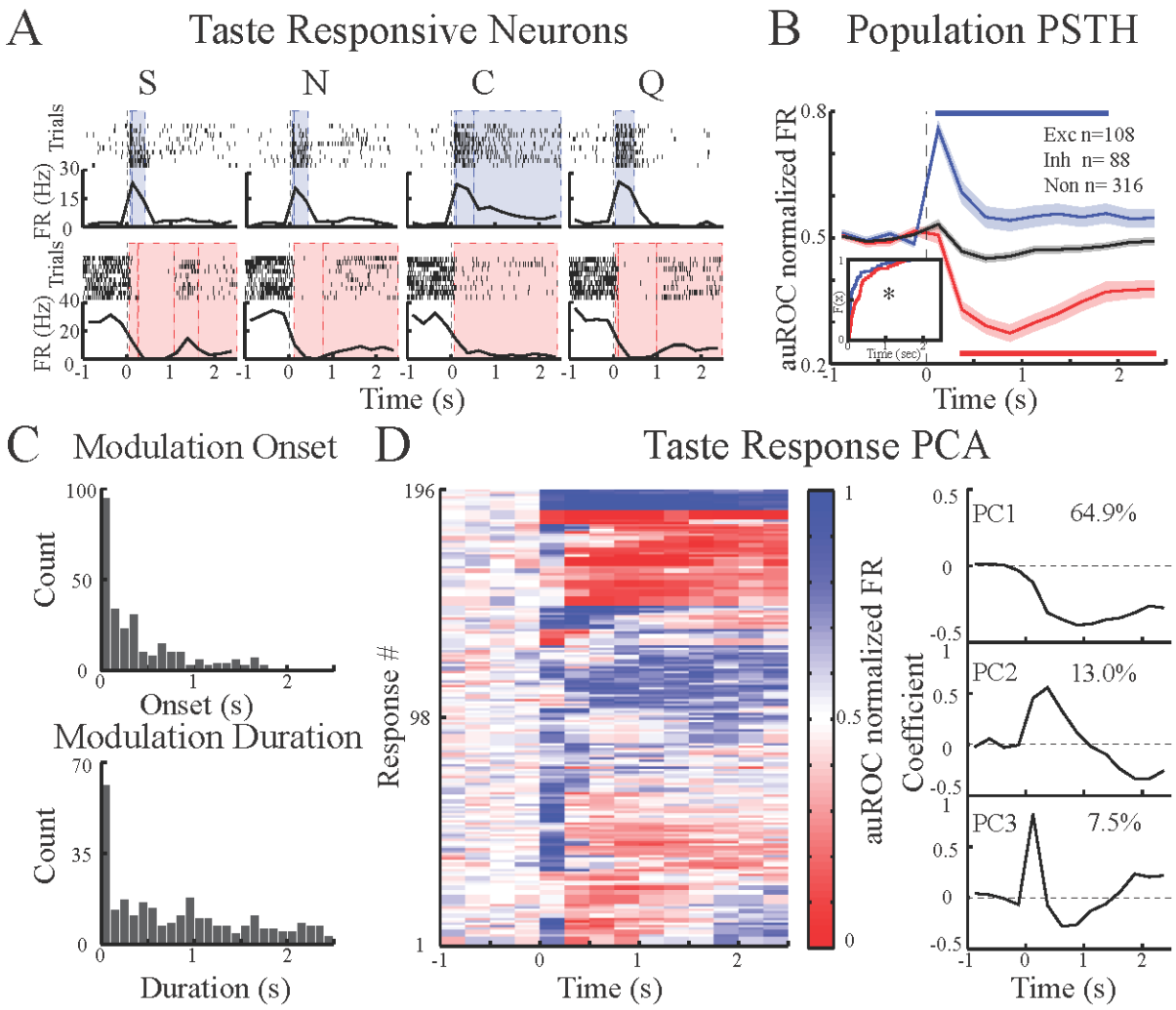

A
Taste Responsive Neurons
S
N
C
Q
Trials
FR (Hz)
Time (s)
B
Population PSTH
auROC normalized FR
Exc n=108
Inh n= 88
Non n= 316
Time (s)
C
Modulation Onset
Count
Onset (s)
Modulation Duration
Duration (s)
D
Taste Response PCA
Response #
Time (s)
auROC normalized FR
Coefficient
PC1
64.9%
PC2
13.0%
PC3
7.5%

Figure 2.2: Responses to taste stimuli are time-varying. A. Representative examples of neurons responding to passive deliveries with increases (top row) or decreases (bottom row) in firing rates. For each neuron, raster plots (top) and PSTHs (bottom) are shown in response to sucrose (S), NaCl (N), citric acid (C) and quinine (Q). Time 0 represent the onset of taste delivery. Bin size is 250 ms. Shaded areas indicate periods in which firing rates are significantly modulated relative to baseline (blue, excitatory; red, inhibitory). Dashed lines represent onsets and offsets of different modulations. B. Population PSTH of excitatory (blue), inhibitory (red), and non-significant (black) responses to taste stimuli. Shaded areas indicate SEM. Solid lines above and below traces indicate the period in which responses are significantly different from those in unresponsive neurons (t-test, $p < 0.05$). The inset shows a cumulative density function of response latencies for excitatory (blue) and inhibitory (red) responses. Asterisk indicates a significant different between the two distributions (Kolmogorov-Simirnov test, $p < 0.05$). C. Distribution of modulation onsets (top) and modulation durations (bottom). D. Identification of response patterns with principal components analysis (PCA). Left: pseudocolor plot of auROC normalized responses to taste. Time 0 is when the stimulus is delivered. Right: Eigenvectors shown for the first three principal components. Top: monophasic component accounting for 64.9% of variance; middle: biphasic component accounting for 13% of variance; bottom: triphasic component accounting for 7.5% of variance.

from neuronal ensembles instead of single neurons led to higher performance. Using the entire group of 56 taste-specific neurons recorded yielded a correct performance 0.70 with a 95% confidence interval (CI: [0.50 0.83]). To determine the size of the ensemble needed for a highly accurate performance (i.e. 95% correct), a surrogate population of thalamic neurons was created (Jezzini et al. 2013; Rigotti et al. 2013). The minimal number of VPMpc-like neurons needed to reach 95% correct classification was 145.

Given that the time course of thalamic responses contained significant information regarding taste identity, additional analyses were performed to further analyze the temporal evolution of taste coding. ANOVA-based and classification-based analyses were performed using a moving window (250 ms window, 50 ms step) on the 2.5 s long period following taste delivery. Figure 2.3E shows the proportion of taste-specific units that passed a one-way ANOVA test ($p < 0.05$, with Bonferroni correction) at each time point. This analysis showed the number of neurons which were taste-specific at each time point following taste delivery. A significant number of neurons in VPMpc became taste-specific after the 3rd bin (i.e., in the interval between 100 and 350 ms) following passive taste delivery. The largest number of taste-specific neurons occurred at ~ 0.5 s, and taste specificity continued to be above baseline for most of the 2.5 s window examined. As control, the same analysis was applied to neurons that were not identified as taste-specific by the two-way ANOVA method (Figure 2.3E, Others). As expected, this group showed very little taste specificity over time. The two histograms were significantly different (two-proportion test, $p < 0.05$) for most of the 2.5 s period examined. Figure 2.3F shows results from a moving-window decoding analysis based on the virtual ensemble of all taste-specific neurons recorded. This analysis revealed the time course of the ensemble-based classification averaged across tastants. Also in this case, taste coding showed an early onset, (i.e. classification became significantly above chance starting from the 3rd bin - i.e., in the interval between 100 and 350 ms – following taste delivery), approached a peak at ~ 0.5 s and lasted to the end of the temporal window analyzed.

2.2.3. Processing of Taste Palatability in VPMpc

Taste identity is not the only feature processed by gustatory circuits. Neurons in GC (Yamamoto et al. 1985b; Accolla & Carleton 2008; Grossman et al. 2008; Sadacca et al. 2012), mPFC (Jezzini et al. 2013), BLA (Fontanini et al. 2009), and other taste related regions (Li et al. 2013; Weiss et al. 2014) have been shown to encode information about taste palatability. Recordings from anesthetized rats suggested the presence of a possible "hedonic tone" in VPMpc as well (Verhagen et al. 2003). Visual inspection of recordings from alert rats confirmed this prediction. Figure 2.4A features a representative example of a VPMpc neuron whose firing activity appears to encode the hedonic value of taste stimuli. Raster plots (top panel) and PSTHs (bottom panel) show a similarity of responses to tastants belonging to the same hedonic category. Indeed, between 250 ms and 1 s, responses to sucrose are similar to those evoked by NaCl, and responses to citric acid are similar to those evoked by quinine. To quantify the number of neurons coding for palatability we computed a palatability index (PI; see 6.2.6) based on a within-neuron analysis of response similarity. A neuron is classified as palatability-coding if it responds similarly to tastants with similar palatability and differently to tastants with opposite

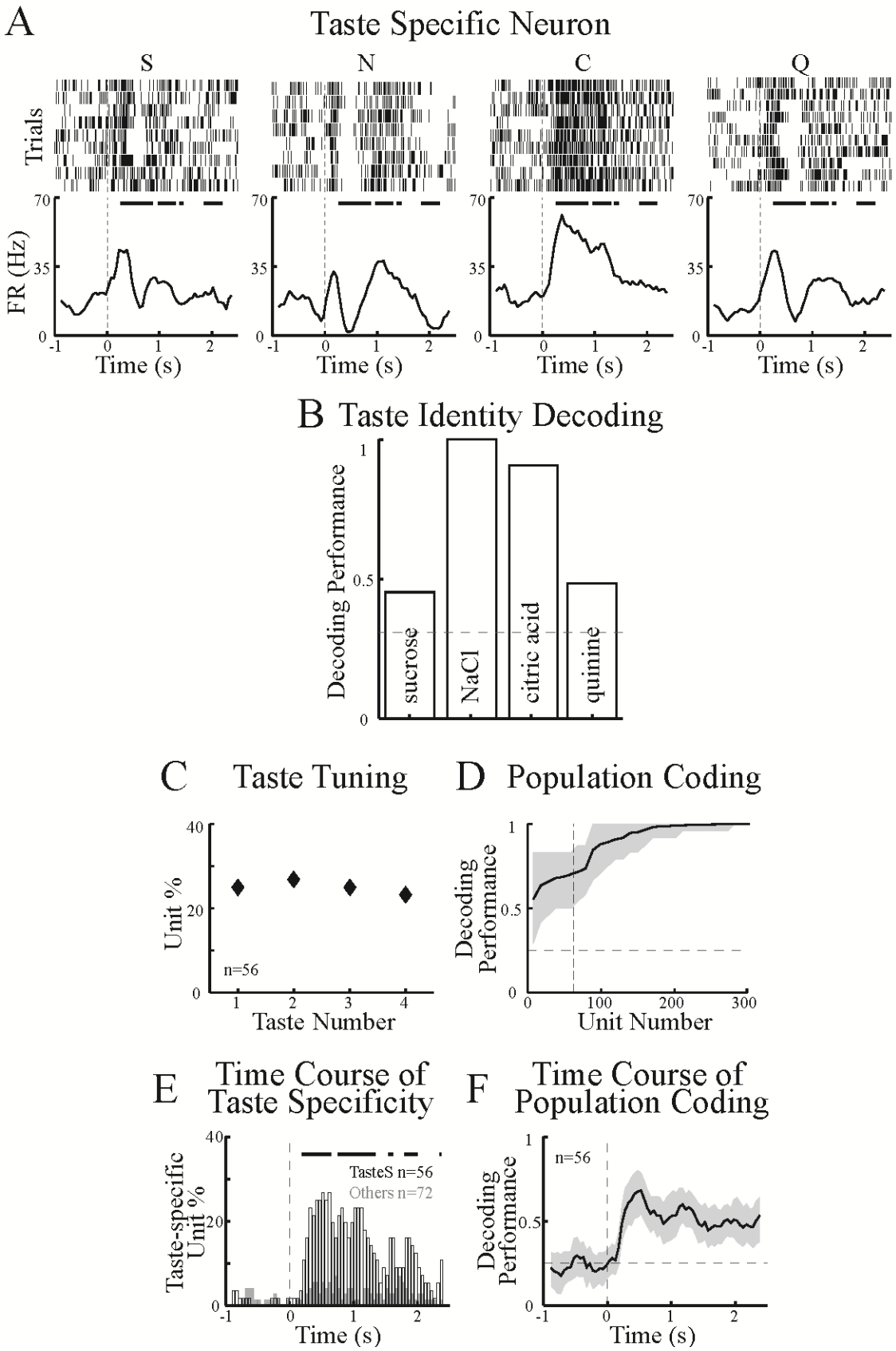

A
Taste Specific Neuron
S
N
C
Q
Trials
FR (Hz)
70
35
0
-1
0
1
2
Time (s)
B Taste Identity Decoding
Decoding Performance
1
0.5
0
sucrose
NaCl
citric acid
quinine
C Taste Tuning
Unit %
40
20
n=56
1
2
3
4
Taste Number
D Population Coding
Decoding Performance
100
200
300
Unit Number
E Time Course of Taste Specificity
Taste-specific Unit %
TasteS n=56
Others n=72
F Time Course of Population Coding
n=56

Figure 2.3: Taste identity coding in VPMpc. A. Representative raster plots and PSTHs for a taste-specific neuron responding to passively delivered S, N, C and Q. Time 0 is when taste delivery occurs. PSTHs are smoothed by a moving window (250 ms, 50 ms step). The thick line above PSTHs indicates times at which responses to the four tastants are significantly different (one-way ANOVA, $p < 0.05$, Bonferroni correction). B. Histogram representing the decoding performance for the representative neuron. The horizontal dashed line indicates the significance level. C. Distribution of units that encode for one, two, three, or four taste qualities. D. Population decoding performance computed on the basis of pseudo ensembles with increasing numbers of units. The horizontal dashed line indicates chance level. Shading represents the bootstrapped CI. The vertical dashed line indicates the ensemble size of recorded taste specific neurons. E. Percentage of taste-specific neurons in the first 2.5 s following taste delivery. Empty bars represent taste-specific neurons (TasteS), gray bars represent neurons that were not taste specific (Others). Time 0 indicates taste delivery. The thick line above the histogram indicates the time points in which the proportion of taste-specific neurons in TasteS group is significantly higher than that in Others group (two-proportion test, $p < 0.05$). F. Time course of decoding performance based on the entire population of taste-specific neurons ($N = 56$). Shading represents the bootstrapped CI. Chance level (0.25) performance is indicated by horizontal dashed line. Time 0 indicates taste delivery.

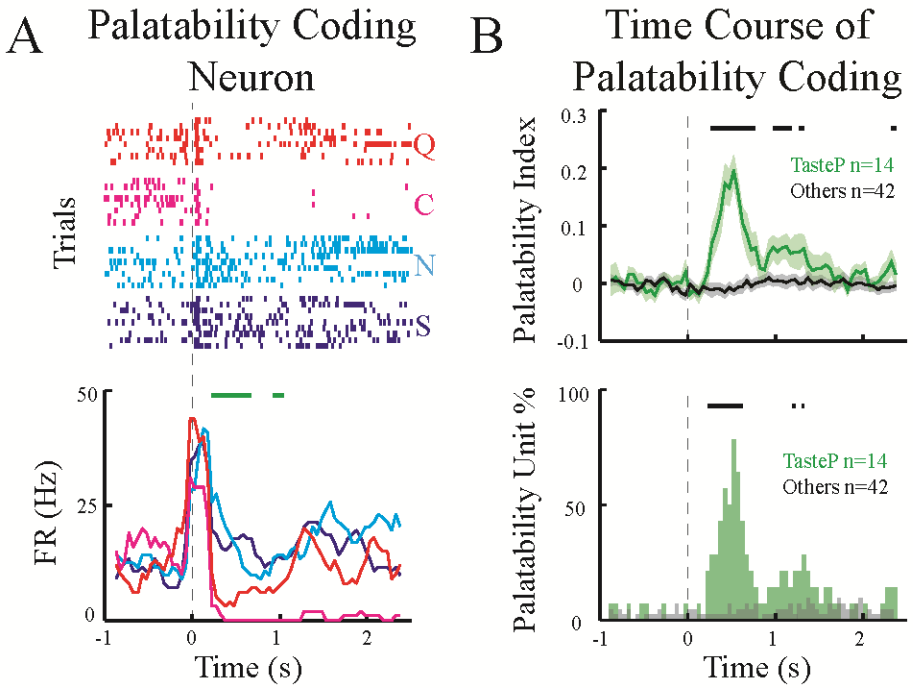


Figure 2.4: Taste palatability coding in VPMpc. A. Raster plots (top) and PSTHs (bottom) for a representative palatability-coding neuron. Responses to S and N (the palatable pair) are excitatory, whereas responses to C and Q (the aversive pair) show a sharp decline followed by inhibition. Green bars represent the time at which the palatability index is significant. B. Time course of palatability coding. Top: analysis of the palatability index over 2.5 s following taste delivery. The green line represents the index computed for palatability-coding neurons (TasteP, $N = 14$). The black line shows the palatability index computed, as control, for neurons that are taste-specific, but not palatability coding (Others, $N = 42$). The thick lines above the traces represent the period in which the palatability index for palatability-coding neurons is significantly above Others (independent-samples t-test, $p < 0.05$). Bottom: time course of the percentage of palatability-coding neurons. Green bars represent the percentage of palatability-coding neurons at a given moment within the palatability-coding neuron group (TasteP); gray bars represent, as a control, the percentage in the group of taste-specific neurons that were not coding for palatability (Others). The thick lines above the histogram indicates the time points at which the proportion of palatability-coding neurons in TasteP is significant higher than that in Others (two-proportion test, $p < 0.05$).

palatability. Neurons were required to have a PI that was significantly above baseline for at least 200 ms in order to be defined as palatability-coding. According to this analysis, 24% of the taste-specific neurons (14/56), 10.9% of total number of neurons recorded (14/128), appeared to be coding for palatability. Recently, a second method was introduced to assess palatability coding (Piette et al. 2012; Sadacca et al. 2012). This analysis relies on the correlation between taste-evoked responses and hedonic ranking (i.e., sucrose > NaCl > citric acid > quinine). We adapted the same method and defined a neuron as palatability-coding if the Spearman correlation coefficient was significant for at least 200 ms. Out of the 56 taste-specific units, 19 turned out to be coding for palatability (33.9%), which accounted for 14.8% of total VPMpc units recorded (19/128).

To determine how coding for palatability evolved in the 2.5 s following taste delivery, the time course of the PI was computed. Figure 2.4B (top) shows that palatability information rises at ~ 0.2 s, peaks at ~ 0.5 s, and tapers down reaching baseline between 1.5 and 2 s. As a control, the PI for taste-specific neurons that were not coding for palatability was plotted and showed no modulation. Computing the number of neurons that were coding hedonic value in each 250 ms bin (50 ms step) following taste delivery revealed a time course similar to the one unveiled by the PI.

Altogether, these results indicate that neurons in the VPMpc of alert rats can encode not only taste quality, but also taste palatability, and that palatability coding emerges soon after taste delivery.

2.2.4. Effects of General Expectation on Taste Coding

Studies in several sensory systems have demonstrated that thalamic responses depend upon the behavioral state of the animal (Cano et al. 2006; Lesica et al. 2006; McAlonan et al. 2008; Saalmann & Kastner 2011; Pais-Vieira et al. 2013). Previous work has identified general expectation as a state strongly affecting taste coding in GC (Samuelsen et al. 2012). To investigate whether gustatory processing in VPMpc varies depending on the general anticipatory state of the animal, we compared responses to self-administered (Self) and passively delivered (Passive) taste stimuli. Comparison of the number of taste-responsive neurons did not reveal any significant difference; 71.9% (92/128) of the neurons recorded responded to Passive and 64% (82/128) to Self (two proportion test, $z = 1.34$, $p = 0.18$) taste stimuli. The proportion of taste-specific neurons was also similar in the two conditions (Self: 63/128, 49.2% vs. Passive: 56/128, 43.7%, two proportion test, $z = 0.88$, $p = 0.38$). However, we observed significant differences in firing rates and taste-response profiles in the two conditions. When analyzing the entire response averaged over 2.5 s, 57% (73/128) of neurons significantly changed their firing rates depending on whether tastants were passively or self-delivered. When the analysis was restricted to neurons that were either taste-responsive or taste-specific in at least one of the two delivery conditions, the proportion of cells that significantly changed firing rates was 63.8% (67 out of 105 taste-responsive neurons in the two conditions) and 69.5% (57 out of 82 taste-specific neurons in the two conditions) respectively. Among the taste-responsive neurons that were modulated by the behavioral state ($N = 67$), 35.82% (24/67) increased, 61.19% (41/67) decreased their firing

activity, and 2.99% (2/67) had mixed effects across the four taste stimuli, in response to Self, compared to Passive deliveries.

In addition to changes in firing rates, neurons changed their response profiles to the four tastants. For this analysis, we compared response profiles with a 3-way ANOVA (taste X condition X time) for neurons that were taste-specific in either one of the delivery conditions (N = 82). 76.8% (63/82) of neurons had significantly different taste tuning depending on the condition. Additional analyses of single neuron ability to encode for single or multiple tastants were performed on taste-specific neurons using a classification algorithm. We observed significant differences in the number of taste qualities encoded by each neuron. In the case of Passive deliveries, an equal proportion of neurons classified above chance for one, two, three or four tastants (24.4% [20/82], 26.8% [22/82], 28% [23/82], and 18.3% [15/82], respectively). In contrast, in the case of Self deliveries neurons classifying only one taste quality represented the minority, and the majority of neurons encoded for four qualities (6.1% [5/82] of neurons encoded for one taste quality, 31.7% [26/82] for two taste qualities, 24.4% [20/82] for three taste qualities, and 37.8% [31/82] for four taste qualities). The two distributions were significantly different (χ^2 goodness of fit test, χ^2 (3, N = 2) = 50.88, p < 10 E -5) (Figure 2.5A). A post hoc comparison revealed that significantly more neurons classified four tastants (two-proportion test, z = 2.78, p < 0.01) and fewer classified one tastant (Fisher exact test, p < 0.01) in Self relative to Passive. Figure 2.5B shows a representative neuron's responses to passively delivered and self-administered tastants. The histogram on the right represents the classification performance for this neuron, which can encode for three stimuli in the case of Self but only one for Passive.

To investigate whether this change in single neuron's ability to represent information about multiple taste qualities impacted taste coding, a series of classification analyses were performed. First, we compared the classification performance for neurons that encoded for one taste quality with that for neurons that encoded for four qualities. In both the cases of Passive and Self, the group of neurons encoding for four qualities allowed for a significantly better average classification performance than that encoding for only one tastant. In the case of Passive deliveries, the average classification performance was 0.31 ± 0.01 for the population encoding only one quality and 0.53 ± 0.02 for the population encoding four qualities (unpaired t-test, p < 0.01). The same was observed for Self deliveries, in which the classification performance was 0.26 ± 0.02 for neurons encoding only one quality and 0.58 ± 0.02 for neurons encoding four qualities (independent-samples t-test, p < 0.01). The results of this analysis suggest that increasing the proportion of neurons encoding for four tastants, and decreasing the proportion of those encoding for one tastant – i.e., the change observed in the case of Self - should result in an overall improvement in taste coding. To test this hypothesis, we averaged the classification performance across neurons and compared it for the two conditions (i.e., Self and Passive). When the best classification performance (i.e., the classification for the "best" taste) was analyzed for each neuron, a higher coding performance was observed in responses to Self than Passive deliveries (0.75 ± 0.02 vs. 0.70 ± 0.02, paired-samples t-test, p < 0.05, N = 82). Furthermore, when the performance of each neuron in classifying all four tastants was computed and averaged, the overall classification was significantly better in Self compared with Passive deliveries (0.47 ± 0.01 for Self vs. 0.40 ± 0.01 for Passive, paired t-test, p < 0.01, N = 82, Figure 2.5C, left). Figure 2.5C right panel shows the percentage of units that significantly decode each of the four tastants, which confirms a higher classification performance for Self (two-way

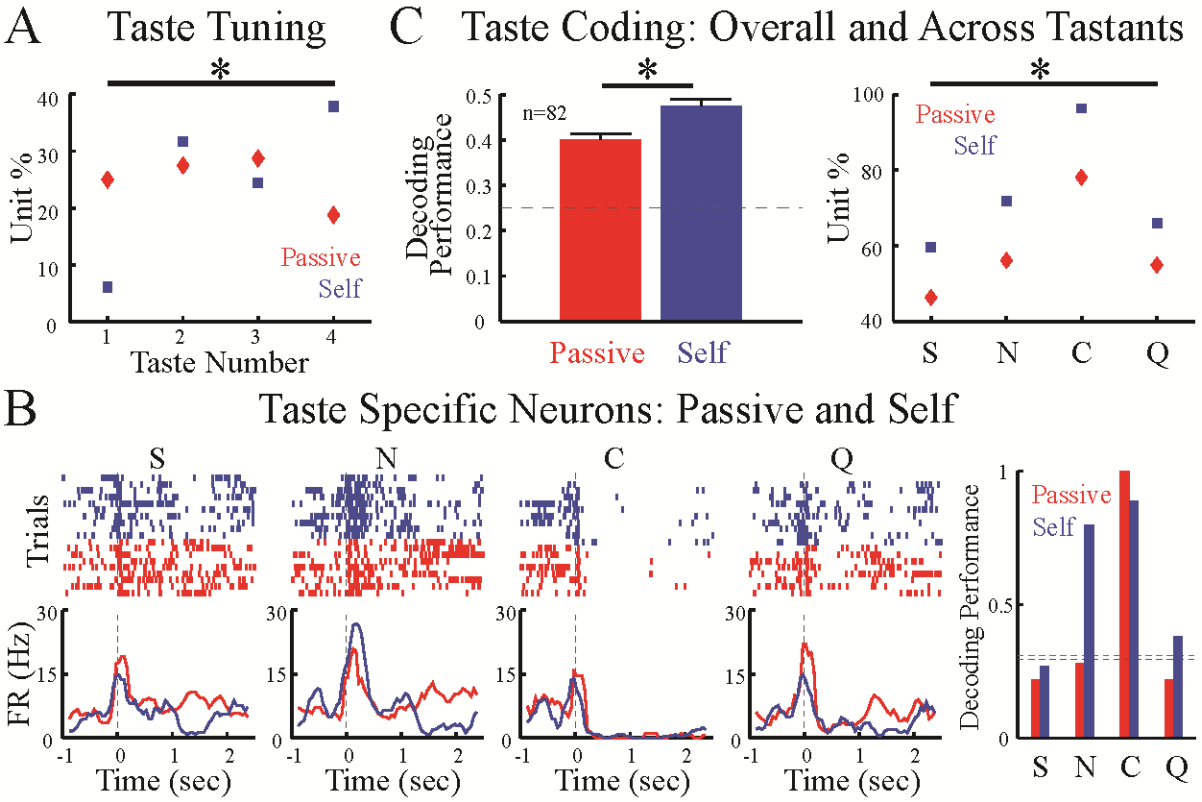


Figure 2.5: State-dependency of taste coding. A. Proportion of neurons that encode one, two, three and four tastants when stimuli are either passively delivered (red, Passive) or self-administered (blue, Self). Asterisk indicates a significant difference between the two distributions (χ^2 goodness of fit test, χ^2 (3, N = 2) = 50.88, p < 10 E -5). B. Raster plots (top) and PSTHs (bottom) for a representative neuron responding to the same stimuli either passively delivered (red) or self-administered (blue). Right: histogram showing the decoding performance for the representative neuron, which encoded significantly just for C in the case of passive and for C, N and Q in the case of self-administrations. Horizontal dashed lines represent significant decoding level. C. Left: decoding performance for passively delivered (red) and self-administered (blue) tastants. Horizontal dashed line indicates chance level. Right: proportion of neurons that decodes for different tastants in the two conditions. Asterisk indicates significance (two-way ANOVA, F(1, 6) = 86.4, p < 0.01).

ANOVA, $F(1, 6) = 86.4$, $p < 0.01$). The improvement of taste coding for self-administered tastants was further confirmed by ensemble-based classification. The population of taste-specific neurons analyzed ($N = 82$) lead to a classification performance of 0.9392 (CI: [0.8333, 1]) for Self and 0.7162 (CI: [0.5417, 0.9167]) for Passive. The size of the population required for accurate classification (i.e., 95%) was 85 in the case of Self, and 185 in the case of Passive deliveries.

Altogether, these results suggest that in the case of self-administered tastants neurons improved their ability to represent multiple taste qualities compared to the Passive condition. This effect resulted in enhanced taste coding.

2.2.5. Effect of General Expectation on Temporal Dynamics

To understand the effects of expectation on the time-course of taste responses, we analyzed response dynamics in neurons that were taste-specific either in Self or Passive conditions (82/128). To quantify differences in firing activity over time, a modulation index (MI) based on differences in auROC was computed (see 6.2.9). The absolute value of MI was used to assess the time at which the largest state dependent change in activity occurred. Figure 2.6A shows that the absolute MI is significantly different from baseline in the entire period examined (-1 to 2.5 s), with a peak at bin 0 to 250 ms. Pre-stimulus increases in MI are likely related to the presence of the cue signaling the time for self-administration. The signed MI was examined to determine if the early difference in responses was biased toward Self or Passive (Figure 2.6B). A bias toward an early reduction of responses to Self was observed, with the largest reduction occurring in the 50 to 300 ms bin. This observation was confirmed by examining the percentage of responses that, in the 50 to 300 ms bin, showed a significant reduction in firing frequency in the case of Self (37.2%, 122/328, of responses showing a reduction with Self compared to 17.7%, 58/328, showing an increase with Self) (Figure 2.6C).

Additional analyses were performed in the group of neurons that were taste-specific either in Passive or Self delivery to determine the impact of these changes on the dynamics of taste coding. First we compared the time course of the percentage of taste-specific neurons for each time bin in the two conditions. This analysis revealed differences starting shortly after stimulus delivery and appearing throughout the 2.5 s period. As shown in Figure 2.7A, significantly more neurons were taste-specific in the case of Self compared to Passive. Interestingly, while only a few neurons were taste-specific in the first two bins (i.e., 0 to 250 ms and 50 to 300 ms) following Passive deliveries, neurons began to show taste specificity as early as the first bin (i.e., 0 to 250 ms) in the case of self-administration. The proportion of taste-specific neurons was significantly larger for Self relative to Passive in the bins: 0 to 250 ms, 50 to 300 ms, and 100 to 350 ms (two proportion test, $p < 0.05$). This result is further confirmed by Figure 2.7B, which shows a significantly shorter latency of taste coding for Self compared to Passive in the subset of neurons for which onset of coding could be detected (Wilcoxon signed rank test, $p < 0.05$, $N = 47$). Analysis of the time course of coding confirmed this result. Both the average of single neuron classification performance (data not shown) as well as the population-based classification performance (Figure 2.7C) revealed a similar phenomenon. The average

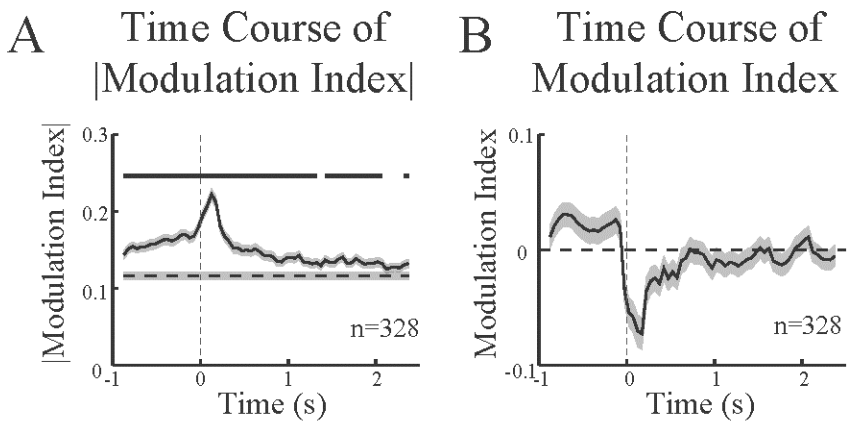
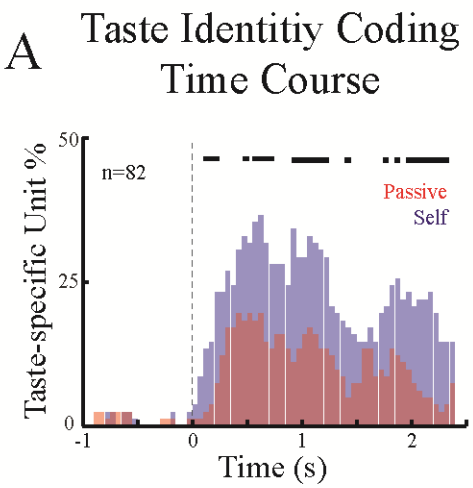
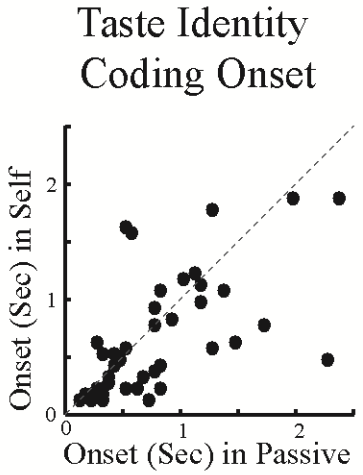


Figure 2.6: Effects of the behavioral state on taste-evoked activity. A. Time course of the absolute modulation index for neurons that were taste-specific in either condition (N = 82 [neurons x 4 tastants]). The dashed line indicates baseline level of modulation index. Shading around the trace represents SEM. The thick line above the trace indicates the time points where the modulation index is significantly above baseline (independent-samples t-test, N = 328, $p < 0.05$). B. Time course of the signed modulation index. Shading around the trace represents SEM. The negative modulation index shortly after time 0 (i.e., taste delivery) indicates that responses to Passive are overall larger than responses to Self.

A


Taste Identitiy Coding
Time Course
Taste-specific Unit %
50
25
0
n=82
Passive
Self
-1
0
1
2
Time (s)

B


Taste Identity
Coding Onset
Onset (Sec) in Self
2
1
0
0
1
2
Onset (Sec) in Passive

C

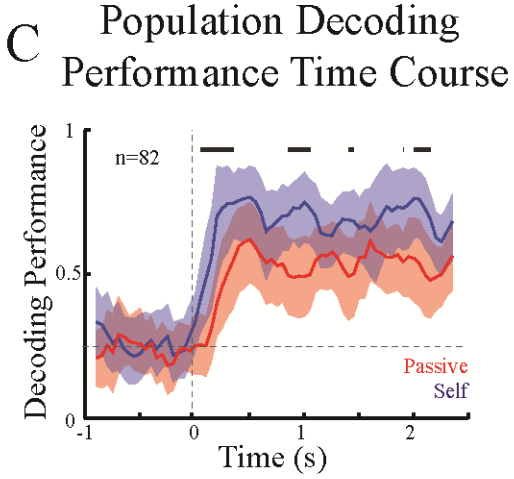

Population Decoding
Performance Time Course
Decoding Performance
1
0.5
0
n=82
Passive
Self
-1
0
1
2
Time (s)

Figure 2.7: Effects of the behavioral state on the time course of coding. A. Time course of identity coding for passively delivered and self-administered tastants. Percentage of taste-specific neurons in the first 2.5 s following passive delivery (red, Passive) or self-delivery (blue, Self). Time 0 indicates the onset of the delivery. Thick lines above histograms indicate the time points at which the histograms for the two conditions are significantly different (two-proportion test, $p < 0.05$). B. Comparison of the onset of taste coding for Passive and Self. Each dot represents the onset of 870 coding for each cell in the two conditions ($N = 47$). C. Time course of decoding performance based on the entire population of taste specific neurons. Red: Passive, blue: Self. Shading around tracing represents the bootstrapped 95% confidence interval. Thick lines above histograms indicate the time points at which the traces are significantly different (bootstrap procedure, $p < 0.05$).

taste classification performance was higher for Self-administration, and the increase in performance showed an early onset, but was not limited to the early phase of the response.

2.3. Discussion

The results presented here provide novel evidence on how the VPMpc of alert rats encodes gustatory information. Multielectrode recordings revealed that intra-orally delivered tastants significantly modify firing rates in a large percentage of VPMpc neurons. Firing rate modulations could be excitatory or inhibitory and were typically time-varying. A combination of tonic and phasic modulations was observed. Around half of the neurons recorded produced responses that were taste-specific and, with the exception of 25% of the neurons, most of the cells encoded for more than one taste quality. In addition to processing the chemosensory nature of tastants, a contingent of neurons in the VPMpc also encoded palatability. Taste identity and palatability are processed in parallel by dynamic modulations of firing rates. The experiments presented here also provide evidence that thalamic coding of taste is state-dependent. Generally expected stimuli, i.e. cued and self-administered tastants, are encoded more effectively and more rapidly than passively delivered tastants. The enhancement in classification performance is related to an increase in the number of tastants being encoded by each neuron.

2.3.1. Thalamic Coding of Passively Delivered Tastants

Approximately half of VPMpc neurons encode the chemical identity of a gustatory stimulus passively delivered directly in the mouth of the animal. This result is in agreement with previous findings in anesthetized rodents showing a similar degree of responsiveness (Verhagen et al. 2003). As a population, taste-specific thalamic neurons encode taste quite effectively. The performance of an ensemble comprising all the taste-specific neurons recorded was 0.70, i.e., tastants were encoded correctly in 70% of the trials. A decoding analysis was performed on virtual populations of thalamic neurons to determine the size of an ensemble capable of highly accurate performance. An ensemble of only 145 neurons was needed to correctly classify taste quality in 95% of the trials.

Analysis of the breadth of tuning revealed that just 25% of the neurons encoded for a single taste quality, when stimuli were delivered unexpectedly via intra-oral cannula. This low degree of selectivity is consistent with a progressive reduction in selectivity observed as gustatory information goes from the periphery to the cortex. In the PbN approximately 40% of neurons were reported to respond exclusively to a single taste quality when tastants were intraorally delivered (Nishijo and Norgren, 1990), although neurons responding to actively licked tastants were recently reported to be more broadly tuned (Weiss et al. 2014). In the case of GC, previous work from our laboratory using intraoral deliveries showed that the percentage of neurons encoding only for a single tastant is limited to 10% (Jezzini et al. 2013). The strong reduction in single taste selectivity from PbN to VPMpc suggests a convergence of parabrachial inputs encoding different single qualities onto VPMpc neurons. A similar convergence can be

envisioned for VPMpc projections to GC. From the functional stand point, the mixed selectivity of VPMpc neurons emphasizes their potential for integrating, rather than segregating, information (Rigotti et al. 2013).

Further evidence that neurons in the VPMpc integrate more than just the physiochemical properties of a single tastant comes from results demonstrating their ability to code for palatability. Recordings in anesthetized rats suggested that neural responses in the gustatory thalamus cluster depended on the hedonic value of the tastants (Verhagen et al. 2003). The data presented here demonstrate this hypothesis in alert rats. Relying on two analyses previously validated for GC, hypothalamus, amygdala and medial prefrontal cortex (Fontanini et al. 2009; Piette et al. 2012; Sadacca et al. 2012; Jezzini et al. 2013; Li et al. 2013), we found that a percentage of taste-specific neurons is responsible for processing palatability.

2.3.2. Temporal Dynamics of Taste Coding and Sources of Information

Identifying the sources of chemosensory and palatability signals to VPMpc is beyond the scope of this investigation. However, an analysis of the temporal dynamics of thalamic responses might provide some hints as to the transfer of information across the PbN-VPMpc-GC axis. Thalamic coding of gustatory information is rapid and persistent. Neurons become taste-specific ~ 225 ms (100 to 350 ms window) after stimulus delivery, reach a peak at ~ 525 ms (400 to 650 ms window) and continue to be taste-specific for at least 2.5 s. While the early onset is likely due to a rapid inflow of information coming from the PbN, the persistency of responses may be the result of recurrent interactions of VPMpc with PbN and GC (Yamamoto et al. 1980; Jones et al. 2006; Sherman 2012). Indeed, reciprocal connections between the gustatory thalamus, PbN and GC are well documented (Allen et al. 1991a; Shi & Cassell 1998; Holtz et al. 2014). The multiphasic changes in firing rates observed with the principal components analysis might in fact reflect waves of information arising from different sources. Reductions in firing rates, either monophasic or multiphasic, are likely to be promoted by inputs from the thalamic reticular nucleus (Hayama et al. 1994).

Similar interactions might be responsible for palatability coding in the thalamus. The time course of palatability coding revealed potentially two palatability epochs, one peaking ~ 450 ms (100 to 800 ms) and the second occurring around one second after taste delivery (800 to 1550 ms). The early phase of palatability coding precedes the palatability epoch observed in GC (Katz et al. 2001; Fontanini & Katz 2006; Jones et al. 2007; Piette et al. 2012; Sadacca et al. 2012; Jezzini et al. 2013) and may depend on inputs from the PbN (Ogawa et al. 1987; Yamamoto et al. 1994; Karimnamazi & Travers 1998; Bester et al. 1999; Krout & Loewy 2000; Soderpalm & Berridge 2000; Holtz et al. 2014). The protracted palatability phase, on the other hand, is likely arising from interactions with GC, where palatability coding has been well-documented.

2.3.3. State Dependency of Thalamic Taste Processing

To investigate the state dependency of thalamic taste coding, we analyzed responses to stimuli that were either passively delivered or self-administered following a cue. This behavioral paradigm was devised to probe the effects of general expectation on taste evoked activity in VPMpc. 57% of the neurons had different responses depending on whether tastants were generally expected or not. Firing rates differed in the two conditions even before taste delivery, likely a result of anticipatory changes in firing (Pritchard et al. 1989; Krupa et al. 2004; Wiest et al. 2010; Samuelsen et al. 2012; Pais-Vieira et al. 2013; Samuelsen et al. 2013). The difference in firing activity peaked shortly after stimulus delivery (between 0 and 250 ms), and lasted up to 2 s. The overall effect was a reduction of firing rates in the case of self-administrations compared to passive deliveries. However, this effect was not dominant, as a large percentage of neurons also showed an increase in firing rates in the case of self-administration. Despite the mixed effects on overall firing rates, self-deliveries were associated with an improvement in taste coding both at the single neuron level and at the population level. Analysis of single neuron taste coding revealed a significant change in the breadth of coding depending on the state of the animal. In the case of passive deliveries, 24.4% of the neurons encoded for only a single tastants and 18.3% for all four tastants. General expectation led to an increase in the ability of single neurons to encode for more tastants; indeed, in this condition, only 6.1% of the neurons encoded for only one tastant and 37.8% for all four tastants. Our results emphasize the advantage of dense representations compared to narrow tuning; a population of neurons encoding for more tastants allows for better taste classification compared to a population of narrowly tuned neurons. In addition, these results indicate that the tuning of gustatory neurons is not fixed and hard wired, but rather dependent on the state of the animal. This evidence is consistent with results from recordings in brainstem and in GC, showing significant differences in tuning for neurons recorded from animals in different behavioral states (Nishijo & Norgren 1990; Fontanini & Katz 2008; Chen et al. 2011; Yoshida & Katz 2011; Samuelsen et al. 2012; Weiss et al. 2014). According to this view, taste coding does not rely on a unique scheme, but is dynamically modulated by the state of the animal.

An additional effect of general expectation was an increase in the speed of taste coding. Indeed, while passively delivered tastants appear not to be coded very effectively in the first 200 ms following stimulus onset, self-administered tastants are encoded as early as in the first 100 ms. Even neurons that had a long latency of taste coding showed a faster onset in the case of self-deliveries. These results dovetail nicely with the data recently reported for GC (Samuelsen et al. 2012), which shows that a similar paradigm accelerates taste coding. Whether the phenomenon observed here for the thalamus is the cause of the effect observed in GC is not known, but it is reasonable to speculate that it might play a role. As for the sources of this modulation in VPMpc, given the presence of well-described cue-responses in GC (Samuelsen et al. 2012), it is reasonable to speculate that cortico-thalamic inputs might play a role in priming VPMpc neurons to receive sensory inputs. In addition, while few data is available for the functions of neuromodulatory projections in VPMpc, it is likely that these inputs might also play a role in modulating VPMpc activity, similarly to what observed for other thalamic nuclei (Hallanger et al. 1987; Sherman 2012; Varela 2014).

2.4. Conclusion

Despite occupying a crucial position in taste pathways, the VPMpc remains a relatively understudied thalamic nucleus. Indeed, little is known on how the VPMpc processes gustatory information in alert rodents. Our results show that VPMpc neurons are broadly tuned to multiple taste qualities and dynamically encode taste quality and palatability. By studying taste coding in two different conditions, we were able to demonstrate that temporal dynamics as well as taste tuning are state dependent and affected by the general expectation of taste. These results demonstrate the importance of taking into account the behavioral state of the animal when investigating coding schemes in taste.

Chapter 3

Anticipatory Signals in the Gustatory Thalamus of Alert Rats

3.1. Introduction

Historically, the gustatory thalamus, i.e., the parvicellular portion of the ventral posteromedial nucleus of the thalamus (VPMpc), has been viewed as a passive relay for chemosensory and orosensory information (i.e., tactile, thermal) (Norgren & Lundy 1995; Scott & Erickson 1971; Ogawa & Nomura 1988; Verhagen et al. 2003). However, my work and recent studies in other sensory thalamic nuclei have shown that the thalamus can do more than just encoding sensory information. For example, studies have found that thalamic neural responses differ substantially across different animal states (Weyand et al. 2001; Swadlow & Weyand 1985; Li et al. 1999; Bezdudnaya et al. 2006; Cano et al. 2006; Fanselow & Nicolelis 1999; Fanselow et al. 2001). Activity in the thalamus can be modulated by attention, expectation, and reward (O'Connor et al. 2002; Ling et al. 2015; McAlonan et al. 2008; Pantoja et al. 2007; Pais-Vieira et al. 2013). In the previous chapter, I have shown that VPMpc also encodes taste palatability and its taste coding is affected by the psychological state of the animal. Specifically, I demonstrated that taste coding in VPMpc is improved by expectation.

Previous evidence suggests that VPMpc might be involved in processing taste anticipation. Behavioral studies of the gustatory thalamus have shown that, although VPMpc is not required for low-order functions such as taste detection and recognition (Reilly 1998; Reilly & Pritchard 1996a; Scalera et al. 1997), VPMpc lesions impair complex, taste-related behaviors (Reilly et al. 2004; Reilly & Pritchard 1997; Reilly 1998; Schroy et al. 2005; Reilly & Trifunovic 2003). For instance, after associating a palatable saccharin solution with the subsequent, more preferred sucrose solution, rats will reduce the intake of the saccharin solution. This phenomenon is called anticipatory negative contrast (ANC) (Reilly & Trifunovic 2003). VPMpc lesions were found to disrupt ANC (Schroy et al. 2005; Reilly et al. 2004). This suggests that VPMpc might participate in higher-order functions such as anticipation. Moreover, a recording study from alert monkeys has observed that a group of neurons in VPMpc modulated their firing rates before receiving anticipated water rinse (Pritchard et al. 1989). Due to the limitation of the experimental design, anticipation-like activity was confounded by concurrent preparatory motor movements (Pritchard et al. 1989). Recently, data from our lab (Samuelsen et al. unpublished results, Figure 1.5) suggested a possible contribution of VPMpc to taste anticipation in GC. In this experiment, the authors recorded GC neural activity while inactivating VPMpc after rats had associated an auditory cue with taste deliveries. Inactivation of the VPMpc had a small effect on the overall magnitude of cue responses in GC, as computed by averaging across all the cue responsive

neurons (Samuelsen et al. 2013). However, analysis of single neuron activity revealed that VPMpc inactivation changed the temporal dynamics of cue responses in ~70% of cue responsive neurons in GC. This work suggests that VPMpc might respond to cue presentation and modulate GC cue responses.

While previous studies have hinted a potential role of VPMpc in taste anticipation, a convincing evidence was lacking. Here, we present results from single unit recordings in behaving rats unveiling that VPMpc processes taste anticipatory information. In this study, we trained rats to self-administer taste solutions by entering a nose port after an auditory cue. We found that a group of VPMpc neurons showed significant modulation when the cue was played. However, isolated auditory stimuli (i.e., tones at frequencies different from the cue and not paired with taste) rarely activated VPMpc neurons, suggesting that cue responses in VPMpc are based on the cue-taste association. By monitoring mouth movements with electromyographic (EMG) recording, we ruled out motor-related confounding factors of cue responses. Relating cue responses with behavioral output allowed us to determine that the strength of cue responses depended on the state of the animal and predicted its behavioral responses. To our knowledge, these results provide the first electrophysiological evidence that neurons in the sensory thalamus process anticipatory information by responding, in a state-dependent fashion, to anticipatory cues.

3.2. Result

One hundred and twenty eight single units were recorded from the VPMpc of eleven rats. Electrode positioning was verified with *post hoc* histological reconstruction of recording tracks. Only recordings from electrodes passing through the VPMpc were used for analyses. Data regarding histology and taste quality coding were described in the previous chapter.

To study taste anticipation, subjects were tested after being trained to perform a behavioral task. Each trial started with an auditory cue (pure tone, 80 dB, 0.5 s). Within 3 s after tone termination (i.e., response window), rats had to enter a nose port in order to self-administer taste solutions via intro-oral cannula (IOC). The cue signaled the availability of taste in a general form, as taste solutions were chosen randomly from four classical tastants (sucrose, NaCl, citric acid, or quinine). In order to assess taste responsiveness to passive taste deliveries, taste solutions were also delivered during inter-trial intervals (ITI, ~ 40 s). Water was used as rinse and delivered 5 s after each taste delivery.

3.2.1. VPMpc Neurons Respond to Anticipatory Cues

To assess whether VPMpc neurons process taste anticipation, we analyzed neural activity triggered by the cue in successful trials (i.e. trials in which rats responded with a port entry within the response window). 64% (82/128) of recorded neurons showed significant modulation during tone period (0.5 s) relative to baseline activity (change point method, see 6.2.2). Both excitatory (35.9%, 46/128) and inhibitory (28.1%, 36/128) responses were seen. Figure 3.1A shows a representative example featuring a phasic excitatory modulation during the cue; and

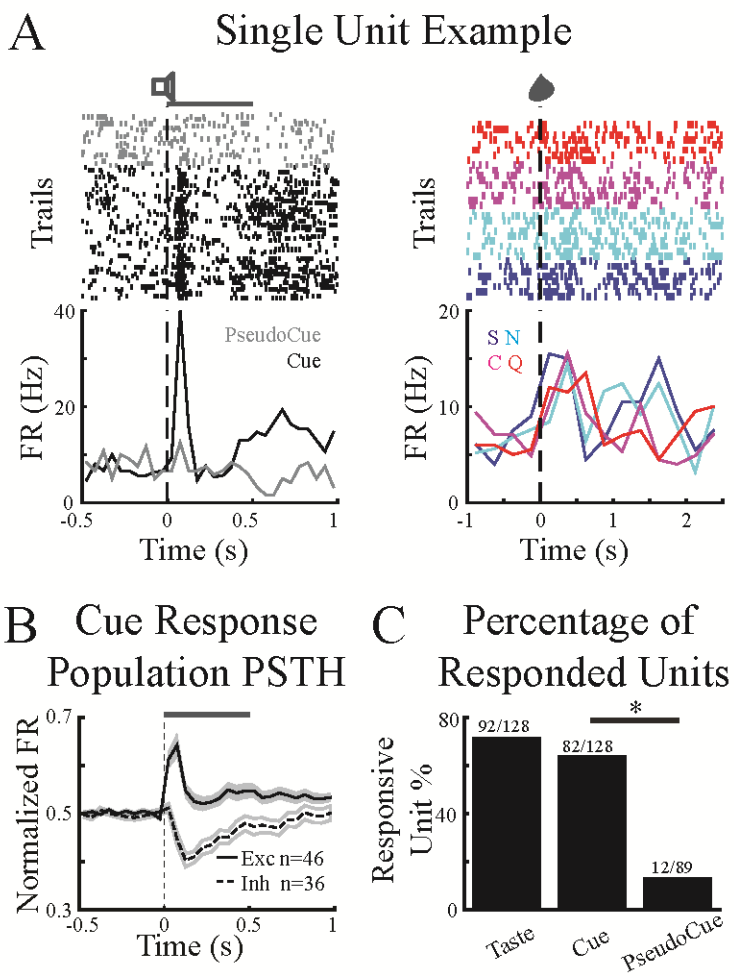


Figure 3.1: VPMpc neurons respond to taste-predictive cues. A. Raster plots and PSTHs of a representative neuron. Left: excitatory response is elicited by an auditory cue predicting the availability of taste (black), while the pseudo cue (i.e., a sound not associated with any taste delivery) does not modulate neural activity (gray). Black bar indicates the period during which the cue is on (0.5 s). Right: neural responses evoked by taste (S - sucrose; N - NaCl; C - citric acid; Q - quinine). B. Population PSTHs of excitatory (solid line) and inhibitory (dash line) responses to the cue. Shaded area and box indicate SEM. Black bar indicates the cue period. C. Percentage of untis that respond to: taste stimuli, cue, and pseudo cue. Black bar and asterisk indicate significance (two proportion test, $z = 7.40$, $p < 10$ E -5).

population PSTHs are displayed in Figure 3.1B. Excitatory responses exhibited slightly earlier onset, though not significantly, compared with inhibitory responses (excitatory response latency: 66 ± 12 ms; inhibitory response latency: 78 ± 14 ms; two-sample Kolmogorov-Smirnov test, p = 0.077). Taste responsiveness was analyzed on the basis of passive taste deliveries. 72% (92/128) was identified as taste responsive (Figure 3.1C). The majority of cue responsive neurons (82%, 67/82) were also taste responsive. Therefore, VPMpc neurons respond to the cue that signals taste anticipation, as well as process taste.

To determine whether cue responses depended on the predictive value of the cue, we introduced, in a subset of animals (N = 6), a second auditory stimulus. The second stimulus, a pure tone having a frequency different from the cue, was not paired with any taste delivery. As such, it was considered a "pseudo-cue". Since the pseudo cue was not reinforced, it did not evoke as many behavioral responses as the cue did (correct response ratio: cue, 72 ± 2 %; pseudo cue, 26 ± 2 %; paired-sample t test, p < 10 E -5). When examining neural activity triggered by the pseudo cue, cue responsive neurons rarely showed significant modulations. Figure 3.1A compares the activity of one representative cue responsive neuron between cue (black) and pseudo cue (gray). While cue evoked a strong phasic response, neural activity during pseudo cue was not different from its baseline. In all neurons tested with pseudo cues, only 13% (12/89) showed significant modulations, a value significantly lower than the percentage of neurons modulated by the cue (cue, 82/128 vs. pseudo cue, 12/89, two proportion test, z = 7.40, p < 10 E -5). This experiment allowed us to conclude that cue responses in VPMpc are specific to taste-predicting cues.

3.2.2. Cue Responses are not Due to Mouth Movements

It is well-known that conditioned stimuli can trigger conditioned behavioral responses (Domjan 2004; Domjan 2014). In the case of gustation, taste-predicting cues can trigger conditioned movements of the mouth (Samuelsen et al. 2013; Samuelsen et al. 2012). As VPMpc neurons can respond to the tactile stimulation of the oral cavity (Nomura & Ogawa 1985; Verhagen et al. 2003), mouth movements triggered by the cue could potentially affect neural activity in VPMpc. Indeed, anticipation-like activity could not be disambiguated from concurrent preparatory motor movements and somatosensory activity in the aforementioned study in monkeys (Pritchard et al. 1989).

To rule out potential confounding factors of mouth movement on the coding of taste anticipation, we recorded and analyzed the EMG activity of mouth muscles simultaneously with VPMpc neural activity in a small subset of animals (N = 2). Custom-made recording electrodes were implanted in the anterior digastric muscle (jaw opener), whose EMG signal faithfully tracks movements of the mouth (Travers et al. 1987; Travers & Norgren 1986). Indeed, mouth movements caused by taste deliveries were represented by large amplitude responses in EMG traces (Figure 3.2A). To determine mouth movement onset, we defined a detection threshold (mean + 3 × standard deviation) based on activity during the baseline period (0.5 s before cue onsets or passive taste deliveries). The onset of mouth movements was defined as the first time point where the average EMG signal exceeded the threshold (Figure 3.2C). When comparing cue response latencies with mouth movement onsets, cue response onsets occurred much earlier (Cue: 72 ± 18 ms; Mouth: 517 ± 68 ms; paired-sample t-test, p < 10 E -5, N = 21; Figure 3.2D).

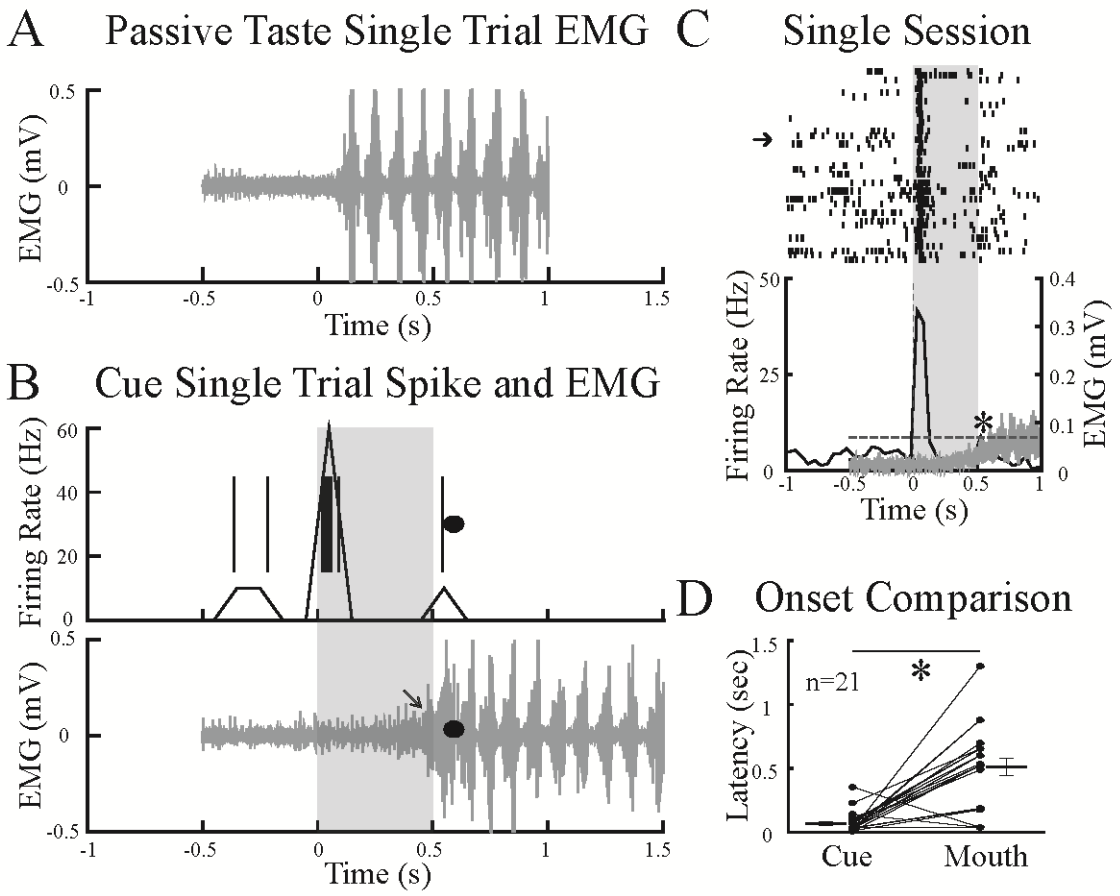


Figure 3.2: Mouth movements happen later than the cue response onset. A. Single trial example of raw EMG traces in response to a passive taste delivery. B. Single trial spike and EMG to cue. The raster and PSTH plots of the cue response (upper) and the EMG trace (lower) are recorded simueltaneouly. The black dot indicates when the taste is self-administered; shaded box indicates the cue period (0.5 s); arrow indicates the conditional mouth movement onset detected by visual inspection of the EMG trace. C. A representitive single session example. Raster and PSTH of the neuron shown in B (black) are overlaid with the average of rectified EMG trace (gray). Dash line indicates the mouth movement detection threshold determined based on baseline (-0.5 - 0 s); asterisk indicates mouth movement onset. Arrow indicates the trial shown in A. C. Comparison between cue response onsets and mouth movement onsets across multiple sessions and animals. The black bar with asterisk indicates significance (paired-samples t-test, $N = 21$, $p < 10$ E -5). The black lines with error bars indicate mean and SEM.

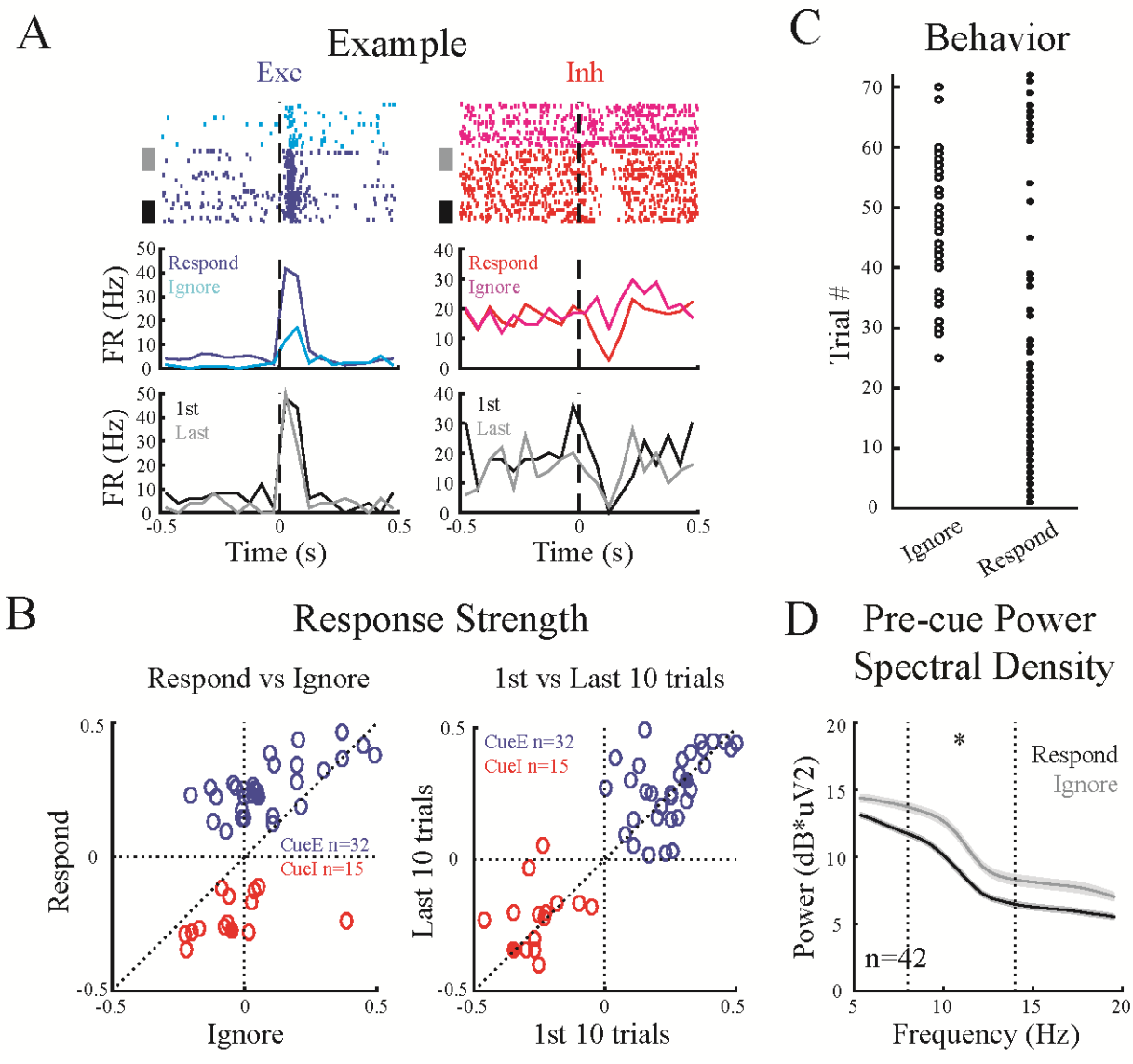
This result was further confirmed by performing single-trial visual inspection. The session means of single-trial mouth movement onsets were similar to the onsets identified by the threshold method for cues (visual inspection method: 574 ± 56 ms; detection algorithm: 517 ± 68 ms, paired-samples t-test, p = 0.53, N = 13) and for passive taste deliveries (visual inspection method: 68 ± 2 ms; detection algorithm: 51 ± 16 ms; paired-samples t-test, p = 0.35, N = 13). Comparison between the earliest onsets by visual inspection (213 ± 23 ms, see 6.2.12) and cue response onsets (72 ± 18 ms) also indicated that cue responses occurred earlier (paired-samples t-test, p < 10 E -5, N = 21). Therefore, our results demonstrated that cue responses in VPMpc are not due to conditioned mouth movements, but encode taste anticipation.

3.2.3. The Strength of Cue Responses Correlates with the Behavioral Outcome to Cues

In our task, rats were trained to allow for performance variability. First, no punishment was given if rats missed a trial. Second, water deprivation was only at a moderate level (post-session supplementary water access was provided, see 6.1.4). Under these conditions, rats showed 3 types of behavioral reactions to the cue: 1) correct go within the response window (3 s); 2) late go response (port entry after the 3-s long response window); 3) no go (rats did not show any tendency to respond to the cue). Of these three type of trials we restricted our analysis to trial type 1 (i.e., correct go; defined hereafter as "Respond") and trial type 3 (i.e., no go [no port entry within 6 s after the termination of the cue]; defined hereafter as "Ignore"). These two scenarios represented animals' decision to react to the cue. We identified the sessions that showed at least 10 trials for each trial type for further analyses. The identified sessions (N = 42) contain 32 cue-excited and 15 cue-inhibited neurons.

Cue-evoked activity exhibited substantial differences between the two trial types. In the examples shown in Figure 3.3A, cue responses were reduced for both the excitatory and inhibitory modulations, i.e., less excited and less inhibited, in the "Ignore" trials compared with the "Respond" trials. To quantify the response strength, we first normalized neural activity during the response period according to its time-length-matched baseline period using ROC method (see 6.2.1). We then subtracted 0.5 from the normalized firing rates. Thus, the response strength spanned [-0.5, 0.5], with 0 meaning baseline level and positive (or negative) meaning excitatory (or inhibitory) responses. We found the majority of neurons showed a reduction of response strength in the Ignore trials, when compared with that in the Respond trials (excitatory responses: Respond 0.26 ± 0.02 vs. Ignore 0.08 ± 0.03, Wilcoxon signed-rank test, p < 10 E -5; inhibitory responses: Respond -0.23 ± 0.02 vs. Ignore -0.06 ± 0.04, Wilcoxon signed-rank test, p < 10 E -5). This was illustrated by plotting the response strength in the Ignore trials in the x axis and that in the Respond trials in the y axis (Figure 3.3B). Most neurons with excitatory cue responses (blue circles) fell on the upper-left side of the diagonal line (28 of 32) and all neurons with inhibitory cue responses (red circles) fell on the lower-right side of the diagonal line (15 of 15) (Figure 3.3B, left).

Different from the Respond trials, which were distributed throughout the session, the Ignore trials tended to happen in the latter half of the session (Figure 3.3C). To rule out the possibility that the time passing in the task affected neural responses, we compared the 1st and last 10 trials in the Respond trials (the whole session lasted ~ 45 min). As shown in Figure 3.3A, cue responses of the 1st and last 10 trials mostly overlapped. There was no clear direction of


A
Example
Exc
Inh
Respond
Ignore
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Time (s)
C
Behavior
Trial #
Ignore
Respond
B
Response Strength
Respond vs Ignore
1st vs Last 10 trials
CueE n=32
CueI n=15
Respond
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Last 10 trials
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Pre-cue Power Spectral Density
Power (dB*uV2)
Frequency (Hz)
n=42
*

Figure 3.3: Cue response strength predicts behavior and depends on the attentional state of the animal. A. Raster and PSTH plots of representative examples of excitatory (left) and inhibitory (right) cue responses. The response strength is larger in "Respond" trials (dark color) compared to "Ignore" trials (light color). Bottom shows PSTHs for the 1st (black) and last (gray) 10 trials of cue responses in the Respond conditon. Black and gray boxes on the side of raster plots indicate the 1st and last 10 trials. B. Scatter plots comparing the strength of cue responses in the two conditions. Left: the Respond vs Ignore conditions; right: 1st vs last 10 trials in the Respond condition. Each circle represents a neuron and is color coded: excitatory (blue) and inhibitory (red). Filled circles indicate the neurons shown in A. C. Trial sequence in a representative session. Identifed trials (Respond: filled circle; Ignore: open circle) are aligned chronologically. D. Power spectral density of LFP around α band (8-14 Hz) in the pre-cue period. Dot lines indicate α band (8 - 14 Hz); Respond condition: black; Ignore condition: gray; asterisk indicates significance (Wilcoxon rank test, $N = 42$, $p < 0.05$ with Bonferroni correction).

changes between the first and last 10 trials in the response strength (excitatory responses: 1st 0.25 ± 0.02 vs. last 0.27 ± 0.02, Wilcoxon signed-rank test, $p = 0.58$; inhibitory responses: 1st -0.25 ± 0.02 vs. last -0.22 ± 0.03, Wilcoxon signed-rank test, $p = 0.59$) (Figure 3.3B). Similar amount of neurons fell on the two sides of the diagonal line (excitatory cue response: 15 vs 17; inhibitory cue response: 8 vs 7, Figure 3.3B, right). Therefore, the cue response strength correlated with the animals' behavioral response to the cue and not with time passing.

3.2.4. Cue Responses are State Dependent

Besides the different behavioral responses, video observation suggested that the general state of animals was distinct between the Respond and Ignore trials. In the Respond trials, rats waited by the nose port, and responded reliably; while, in the Ignore trials, rats appeared uninterested in the cue and, sometimes, oriented away from the port. This observation suggested that animals might be in different behavioral states before the onset of the cue in the two trial types. Indeed, the distribution of the two types of trials in the behavioral task showed some state-transition-like behavior. As shown in Figure 3.3C, the same type of trials seemed to cluster, i.e. several same-type trials happened in a row. It has been shown that animal states can modulate neural activity in the thalamus and thalamic neurons can show distinct responses to the same sensory stimulus (Fanselow et al. 2001; Cano et al. 2006; Bezdudnaya et al. 2006). Thus, the difference of the cue response strength we observed may be due to different states rats were in. To test this hypothesis, we examined the state of the animal by analyzing local field potentials (LFP) prior to the cue onset in the two trial types. The power spectrum density calculated from the 0.5-s activity before cue onsets showed a significantly higher power in α band in the Ignore trials than that in the Respond trials (Figure 3.3D; Wilcoxon rank test, $p < 0.05$ with Bonforroni correction). It has been shown that α band oscillation (8 - 14 Hz) of LFP in the thalamus is a faithful indicator of attentional levels. High power in α band correlates with low level of attention, and vice versa (Haegens, Händel, et al. 2011; Haegens et al. 2012; Haegens, Nácher, et al. 2011). Thus, rats were inattentive in the Ignore trials with higher power in α band, and attentive in the Respond trials with lower power in α band. The level of attention had a big impact on cue responses, as shown above. Therefore, animal states modulated cue responses and led to different behavioral outcomes to the same cue.

3.3. Discussion

The results presented here provided novel electrophysiological evidence that VPMpc can process taste anticipation. Multi-electrode recordings were performed in rats engaged in a behavioral task. VPMpc neurons were found to respond to the cue that directed rats to self-administer taste solutions. The pseudo cue, which was not reinforced, however, rarely activated VPMpc neurons. Using EMG to monitor mouth movements simultaneously with neural recordings, we ruled out the potential confounding factor due to conditioned mouth movements. Thus, VPMpc neurons encode the anticipation of taste by responding to the taste-predicting cue. Moreover, the strength of cue responses predicted whether rats would respond to or ignore a trial. This modulation of cue response strength was dependent on the attentive state of the animal.

3.3.1. Cue Responses in VPMpc Signal the Anticipation of Taste

Previous studies on the VPMpc focused on processing of orosensory and chemosensory properties of taste and were mostly conducted in anesthetized preparation (Scott & Erickson 1971; Nomura & Ogawa 1985; Verhagen et al. 2003). Inspired by the observation of anticipation-like activity from recordings in VPMpc of awake Macaque monkeys (Pritchard et al. 1989) and by lesion studies revealing the involvement of VPMpc in complex, taste-related behaviors (Reilly 1998; Schroy et al. 2005; Reilly & Trifunovic 2003), we designed a systematic approach to investigate taste anticipation in VPMpc of alert rats. In the current study, we used a pure tone as the sole predictor of taste availability. The tone was salient (80 dB) and short (0.5-s long), such that expectation could be precisely triggered in a temporally defined window. Rats were trained to respond by port entry after tone offset, thus, behavioral reactions did not contaminate the cue window. Using this experimental design, we found that a considerable group of neurons (64%) in VPMpc showed significant modulations during the cue. Cue responses were fast with a mean latency shorter than 100 ms. Both excitatory and inhibitory modulations were seen (Figure 3.1B).

To exclude potential confounding factors due to sensory aspects of the cue or conditioned mouth movements, we performed several control experiments. First, for a subset of animals, we played another tone in the session. Responding to the other tone did not lead to any reward, so that the other tone did not gain associative value and was called pseudo cue. The cue and the pseudo cue only differed by their frequencies and were counterbalanced across animals. When examining neural activity triggered by the pseudo cue, only a very small group of neurons (13%) showed significant modulations. The percentage is much smaller than that of cue responsive neurons (Figure 3.1C). Thus, cue responses are specific to taste-predicting tones.

Second, we performed EMG recordings in anterior digastric muscle (jaw opener) simultaneously with VPMpc neural recordings, in a subset of animals. The EMG recording faithfully tracked mouth movements (Travers & Norgren 1986). Consistent with previous reports (Samuelsen et al. 2012; Samuelsen et al. 2013; Gardner & Fontanini 2014), taste deliveries evoked fast mouth movements (Figure 3.2A) and cues evoked conditioned mouth movements before self-administrations. However, our analyses on EMG recordings showed that any detectable mouth movement happened after cue response onsets (Figure 3.2). Thus, cue responses in VPMpc are not secondary to motor-related modulations.

The results presented above reveal that VPMpc neurons are capable of responding to taste predicting cues. These responses are cue specific and are not due to motor movements. These results imply that cue responses in VPMpc encode the anticipation of taste.

3.3.2. The Strength of Cue Responses Predicts Animals' Decision

We analyzed two trial types featuring different behaviors: the "Respond" trials, in which rats responded correctly, and the "Ignore" trials, in which rats ignored the cue. Ignoring the cue was correlated with a reduction in response magnitude for the majority of cue responses (Figure 3.3A and B). In addition, we noticed that Ignore trials were more likely to happen in the latter half of the session. To rule out the potential effects of time passing (i.e., nonstationary effects),

we compared the strength of cue responses in the Respond trials at the beginning of a session and at the end (Figure 3.3). No significant trend was observed among cue responsive neurons. These results suggest that the strength of the cue response correlates with the behavioral decision to the cue.

The strong correlation between the cue response and the animals' decision suggests that the observed cue responses could be responsible for motivating behavior. Since anatomical studies have shown that VPMpc projects to both the gustatory cortex and amygdala (Nakashima et al. 2000) and both regions are shown to be important for reward associated behavior (Cardinal et al. 2002; Kusumoto-Yoshida et al. 2015; Schoenbaum et al. 2003), it is reasonable to expect the cue response in VPMpc can have a major influence in these two areas. This result also dovetails nicely with recent findings from our lab. VPMpc inactivation changed the temporal pattern of cue responses in GC (Figure 1.5), which could be resulted from the inactivation of the cue responses in VPMpc. However, whether VPMpc cue responses directly contribute to GC mediated, reward related behaviors (Kusumoto-Yoshida et al. 2015) needs future investigation.

3.3.3. State Dependency of Cue Responses

Neural activity in the sensory thalamus is state-dependent (Weyand et al. 2001; Swadlow & Weyand 1985; Li et al. 1999; Bezdudnaya et al. 2006; Cano et al. 2006; Fanselow & Nicolelis 1999; Fanselow et al. 2001) and can be modulated by the psychological state of the animal, such as attention and expectation (O'Connor et al. 2002; Ling et al. 2015; McAlonan et al. 2008; Pantoja et al. 2007; Pais-Vieira et al. 2013). In this study, our results revealed that cue responses in VPMpc were also state-dependent.

The two distinct trial types (i.e., Respond and Ignore), defined by the behavioral response to the cue, suggested the presence of different behavioral states. Visual observation of animal behavior prior to cue onsets confirmed this speculation. While rats waited by the port before the cue in the Respond trials, they oriented away from it in the Ignore trials. As the power of α band rhythm of LFP in the thalamus is anti-correlated with levels of attention (Haegens, Händel, et al. 2011; Hanslmayr et al. 2011), we analyzed LFP prior to cue onsets. In the Respond trials, the power of α band was lower, compared with that in the Ignore trials. This result suggested that rats were attentive in the Respond trials; and the attentiveness led to stronger cue responses. Meanwhile, inattentiveness in the Ignore trials correlated with weak cue responses (Figure 3.3). The state dependency of cue responses might indicate different perceptions of the cue or different levels of motivation for getting taste, and both of which result in different behavioral outcomes.

3.3.4. The Source of Anticipatory Modulations in VPMpc

Tracking the source for cue responses and their state dependent modulation in VPMpc is beyond the scope of this study. Based on anatomical connections and functional studies, several potential mechanisms can be proposed. First, VPMpc receives massive projections from GC (Maffei et al. 2012; Holtz et al. 2014). The corticothalamic pathway provides diverse and dynamic modulations on neural activity in the sensory thalamus (Briggs & Usrey 2008; Mease et al. 2014; Sillito & Jones 2002). In addition, recent studies have found that GC neurons encode

taste expectations and respond to taste cues (Samuelsen et al. 2012; Gardner & Fontanini 2014). This evidence suggests that GC could drive and modulate VPMpc cue responses. Second, the thalamic reticular nucleus (TRN) innervates VPMpc (Hayama et al. 1994). TRN receives inputs from a variety of cortical and subcortical regions, including sensory thalamic nuclei, sensory cortices, and higher-order regions, such as the prefrontal cortex and amygdala (Zikopoulos & Barbas 2012). Different sections of TRN also interact with each other via electrical and chemical synapses (Shosaku et al. 1989; Cruikshank et al. 2005; Crabtree & Isaac 2002). Thus, TRN provides a circuit for cross-modal and higher-order modulations (Crabtree et al. 1998; Kimura et al. 2007; Crabtree & Isaac 2002; Pinault & Deschênes 1998a; Pinault & Deschênes 1998b). Both inhibitory and dis-inhibitory mechanisms (McAlonan et al. 2006; McAlonan et al. 2008; Crabtree et al. 1998; Halassa et al. 2014) might contribute to the processing of cues in VPMpc. Third, VPMpc also receives projections from neuromodulatory systems, such as cholinergic centers (e.g., laterodosal nucleus [LDT]) and monoaminergic centers (e.g., locus coeruleus [LC]) in the brainstem (Hallanger et al. 1987; McCormick 1989). Stimulating those nuclei has been shown to result in complex effects on thalamic neurons (Devilbiss & Waterhouse 2004; Dossi et al. 1991; McCormick 1989). In addition, LC neurons respond to task cues and exhibit performance dependent modulations (Usher 1999; Aston-Jones & Cohen 2005). These evidence suggest a possible contribution of neuromodulatory innervations for the processing of taste cues in VPMpc.

3.4. Conclusion

Despite occupying a crucial position in the taste pathway, the VPMpc remains relatively understudied. Our results reveal that, in addition to encoding the taste experience in the oral cavity (tactile sensation, taste quality and taste palatability) (Norgren & Lundy 1995; Scott & Erickson 1971; Ogawa & Nomura 1988; Verhagen et al. 2003), VPMpc neurons encode taste anticipation by responding to the cues predicting the availability of taste. These cue responses depend on the attentional levels of the animal and predict the animal's behavioral outcomes.

Chapter 4

Central Role for the Insular Cortex in Mediating Conditioned Responses to Anticipatory Cues

4.1. Introduction

In natural environments, animals use sensory information from various sources to predict the availability of food (Cheng & Newcombe 2005; Garber & Hannon 1993; Stevens 2013). Repeated pairings of a neutral stimulus with the availability of food leads to the formation of associations. Upon association, food-predicting cues become capable of triggering the expectation of food. These expectations drive motivation for food seeking and food consumption (Berridge 2012; Everitt et al. 2003; Petrovich 2011). It is generally believed that cues drive behavior by activating reward-related circuits responsible for coordinating food seeking and food consumption. A large body of evidence shows that regions like the amygdala, ventral striatum, orbitofrontal and prefrontal cortices, and ventral tegmental area can be activated by anticipatory cues (Bissonette et al. 2014; Jodo et al. 2000; Nicola et al. 2004; Schoenbaum et al. 1998; Schultz 2013; van Duuren et al. 2009). Although the reward circuitry involved in cue-triggered, food-related behaviors has been extensively studied, relatively little attention has been devoted to the role of sensory cortical areas in this process. The insular cortex (IC), for instance, has traditionally been studied for its role in the consummatory and postconsummatory phases of feeding (Bermudez-Rattoni & McGaugh 1991; Braun et al. 1972; de Araujo et al. 2006; Oliveira-Maia et al. 2012; Yamamoto et al. 1988). Neuronal ensembles in the IC are involved in taste processing and learning (Balleine & Dickinson 2000; Jezzini et al. 2013; Maier & Katz 2013; T. Yamamoto et al. 1980), and IC function is believed to be limited to the evaluative and sensory aspects of food consumption (Frank et al. 2013; Maffei et al. 2012). More recent evidence, however, has suggested that the IC can also be involved in processing cues associated with food availability or delivery of addictive drugs (Contreras et al. 2007; Hollander et al. 2008; Samuelsen et al. 2012; Tang et al. 2012; Gardner & Fontanini 2014). The presence of neurons that encode for both anticipatory cues and taste suggests a functional integration of reward and expectation processing. The prediction emerging from these studies is that manipulations of IC anticipatory activity might have an impact on food-directed and, in general, reward-directed behaviors. Although pharmacological manipulations of the IC have been shown to have an impact on reward-guided behaviors, no study to date has focused directly on the role of IC cue-related activity in driving those behaviors.

Here, we examined the hypothesis that IC neuronal activity triggered by food-predictive cues modulates behavioral responses to obtain food. Mice were trained in a Pavlovian learning paradigm in which a sensory cue anticipates the delivery of a food pellet. Electrophysiological

recordings of single units were used to investigate whether IC activity tracked anticipation in this task. Pharmacological and optogenetic inactivation of the IC allowed us to determine the behavioral role of IC's anticipatory activity in mediating food port approach response on presentation of the anticipatory cue.

4.2. Results

4.2.1. Conditioned Reward Approach Behavior as an Indicator of Motivation to Food

A classical Pavlovian paradigm was used to induce the anticipation of food in food-restricted mice (Figure 4.1A). A compound visual and auditory cue was associated with the delivery of a food pellet at a receptacle. The cue lasted 11 s, and the pellet was delivered at 10 s. Each session comprised 25 trials, and the intertrial interval (ITI) was variable (45–75 s) such that the animal could not predict the onset of the cue. Mice did not show any response to the cue at the beginning of training; in the first 4 d, the number of food magazine entries during the cue was not significantly different from head entries observed during the ITI. A significant increase in number of entries during cue presentation could be seen after 5 d of training [Figure 4.1B; $F(1,14) = 34.11669$, $p < 10$ E -5 by repeated measures two-way ANOVA (cue vs. no-cue), $p < 0.01$ by Bonferroni's multiple comparisons test at the time point of session day 5]. The emergence of a conditioned response indicates that mice acquired the associative and anticipatory value of the sensory cue. Upon learning of the association (i.e., from day 5), mice showed robust head entry responses during cue presentation in each training day (Figure 4.1B). Averaged head entry activity during trials is shown in Figure 4.1C. There was a significant effect of averaged head entry activity (Figure 4.1C; $F(19,133) = 9.994012$, $p < 10$ E -5 by repeated measures one-way ANOVA). Overall head entry activity was significantly higher than baseline level after 5 s of cue onset (head entry activity in -5 s vs. 5 – 10 s after onset of the reward-predicting cue, $p < 0.05$ by Bonferroni's multiple comparisons test).

4.2.2. Neuronal Activity of Mouse IC During Reward-Predicting Cue

Recent studies using rats have shown that neurons in the IC can change their firing in response to auditory cues anticipating the availability of taste (Samuelsen et al. 2012; Samuelsen et al. 2013; Saddoris et al. 2009). To determine whether IC activity could also be modulated by anticipatory cues in the present experimental conditions, we implanted 14 mice with movable bundles of 8 or 16 electrodes. Recording of single-neuron activity revealed that different phases of a trial could result in significant changes in firing rates relative to precue baseline. Firing rates could either increase or decrease relative to baseline. A total of 35.5% (39 of 110) of the neurons were modulated by the cue (Figure 4.2A, Left), 25.5% (28 of 110) by food delivery (i.e., dropping of the pellet in the last second of the cue; Figure 4.2A, Middle), and 45.5% (50 of 110) by food consumption (Figure 4.2A, Right). Some neurons could be modulated by more than one event [12.7% (14 of 110) for cue and food delivery, 18.2% (20 of 110) for cue and food consumption, 13.6% (15 of 110) for food delivery and consumption, and 7.2% (8 of 110) for all

three]. We focused our attention on the group of cue-modulated neurons. Figure 4.2A (Left*)* shows the population response (normalized as the area under the receiver operating characteristic curve; see 6.2.1) for neurons that were excited [14.5% (16 of 110)] and for neurons that were inhibited by the cue [21.0% (23 of 110)]. The average change in firing rates was 1.22 ± 0.17 Hz relative to baseline firing of 6.33 ± 1.38 Hz [corresponding to a modulation index (MI) of 0.20 ± 0.01; Figure 4.2C] for excitatory responses. Neurons inhibited by the cue showed a reduction of 1.94 ± 0.36 Hz from a baseline firing of 9.67 ± 2.02 Hz (corresponding to an MI of −0.20 ± 0.02; Figure 4.2C). In addition, the peak/trough of cue-evoked activity was computed in the two groups of neurons; peak response was 2.41 ± 1.52 Hz above baseline (corresponding to an MI of 0.16 ± 0.009) for neurons excited by cue. The trough of the response was 3.07 ± 1.73 Hz below baseline (corresponding to an MI of −0.17 ± 0.01) for neurons suppressed by cue. Population activity of cue-modulated neurons did not show strong responses to food delivery or food consumption. This observation suggests that cue modulations do not relate to conditioned mouth movements similar to those mouth movements evoked by food consumption. Figure 4.2B shows raster plots and peristimulus time histograms for two representative units. Additional analyses were performed to determine whether cue-related modulations depended on the act of entering the food port. To track head entry behavior, the rate of head entries was computed for each session. On average, the onset of cue responses was significantly faster than the onset of head entry rate increase (0.73 ± 0.07 vs. 1.88 ± 0.16 s; paired-samples t-test, $p < 10$ E -5), suggesting that cue responses preceded the increase of head entry rate. To investigate the potential contribution of head entries to cue-related modulation further, we compared spiking activity before and after head entries, during both cue and ITI. We applied an unbalanced two-way ANOVA to investigate modulations related to cue, head entries, and the interaction of the two. This analysis revealed that of 110 neurons, 51 showed modulation due to cue, 21 showed modulation by head entries (11 overlapped with the previous category), and only 2 showed a significant interaction term ($p < 0.05$). Then, we focused on the group that showed head entry-related modulation ($N = 21$), and we computed an MI. The absolute MI, a measure of modulation strength, was significantly larger for head entries performed during the cue compared with head entries performed during the ITI (0.11 ± 0.01 vs. 0.05 ± 0.01; paired-samples t-test, $p < 10$ E -5). This result suggests that the cue enhances the head entry-related modulation (if there was any). To support further the evidence that cues are influencing neural activity regardless of head entry, we computed the Fano factor (a measure of neural variability) around head entries during the cue and during the ITI. The Fano factor was significantly smaller when the animal entered the port during the cue, relative to ITI entries (1.44 ± 0.8 vs. 1.63 ± 0.06; paired-samples t-test, $p < 10$ E -4), implying that cues reduced the variability of neural activity preceding head entry.

Altogether, these data show that IC neurons are active throughout different phases of the task, including the anticipatory phase during playback of the cue.

4.2.3. Pharmacological Inactivation of the IC Decreases Food-Oriented Behavior

To examine whether neuronal activity in the IC had an effect on feeding behavior, we tested the effects of pharmacological inactivation of the IC on conditioned responses. We performed local infusions of the GABA_A receptor agonist muscimol and the GABA_B receptor

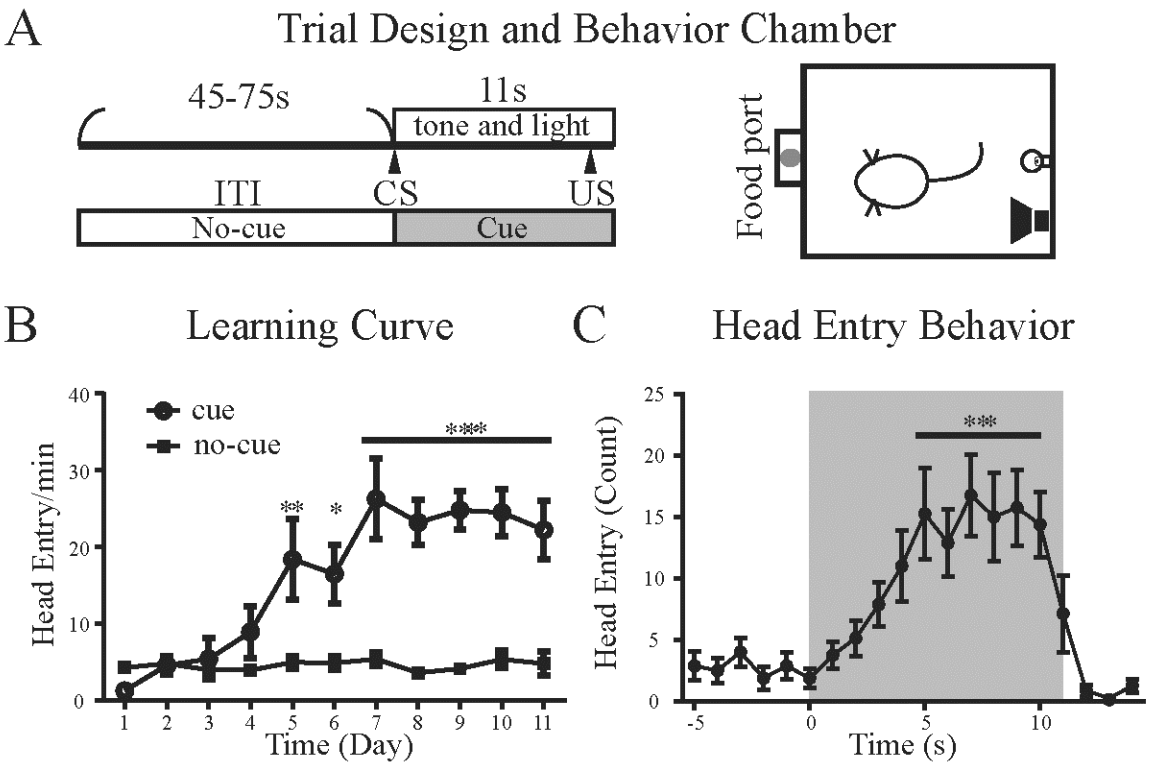

Figure 4.1: Behavioral paradigm used for assessing reward-approach behavior. A. Left: Design of one trial. Each session contained 25 trials. Right: Operant chamber. Food delivery was associated with tone and light presentation. B. Learning of cue-food association and development of food magazine approach behavior. Mean (± SEM) head entry activity across sessions for eight subjects is shown. Head entry activity during the cue presentation period (○) and head entry during the ITI (■) are shown. The x axis represents training days, and the y axis represents averaged head entry activity. C. Mean (± SEM) head entry activity during the pre-presentation, cue presentation, and post-presentation periods (N = 8). Each dot represents the cumulated number of head entries observed in one session. The x axis represents seconds from the onset of the cue, and the y axis represents averaged head entry activity. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 10$ E -4; ****, $p < 10$ E -5.

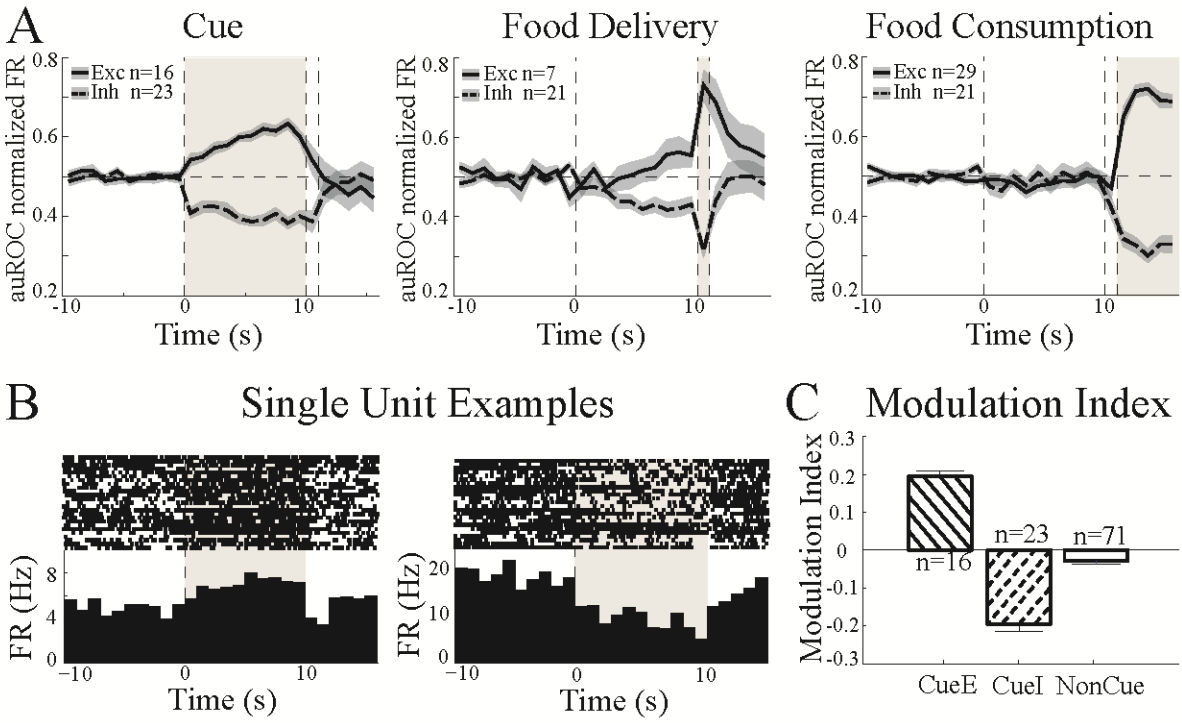

Figure 4.2: Neuronal activity in the mouse IC. A. Population peristimulus time histogram (PSTH) of IC neurons significantly changing their firing in response to the cue (left), to food delivery (middle), and to food consumption (right). Activity is aligned to cue onset (time 0). Solid lines represent excitatory modulations, and dashed lines represent inhibitory modulations. Dark gray-shaded areas indicate SEM. Light gray-shaded areas indicate the cue period (left), the pellet-dropping period (middle), and the 5 s period after cue offset (right), when food pellet is consumed by the animal. The x axis represents time, and the y axis represents normalized firing activity. B. Representative example of excitatory (left) and inhibitory (right) modulations during the cue period. A raster plot and PSTH are shown. Time 0 is the cue onset. Light gray-shaded areas indicate the cue period. The x axis represents time, and the y axis represents firing rates (FR). C. Comparison of MI during the cue period for neurons with excitatory responses (CueE; solid lines, N = 16), inhibitory responses (CueI; dashed lines, N = 23), and not significantly modulated (NonCue; empty, N = 71). Bars indicate SEM.

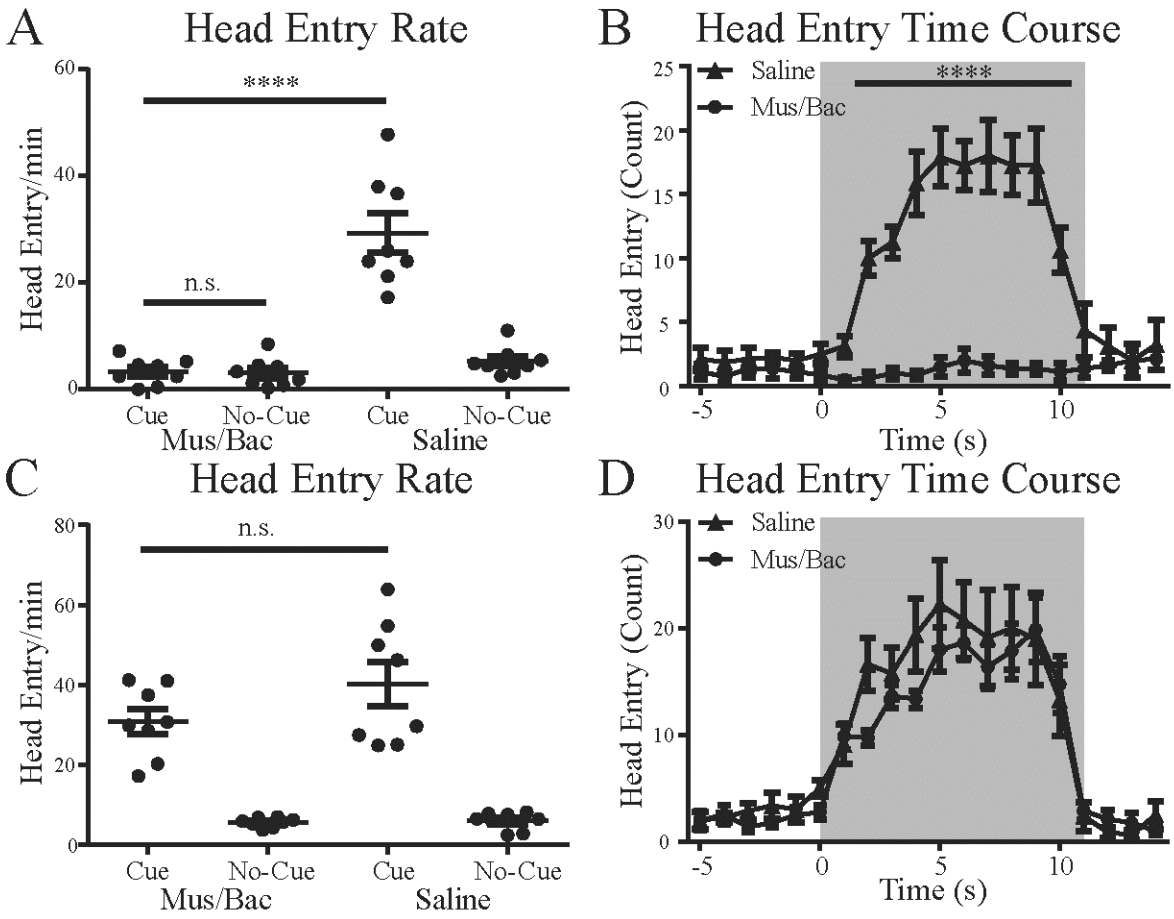

Figure 4.3: Behavioral effects of pharmacological inactivation of the IC. A Head entry responses during the cue period and ITI after injection of muscimol and baclofen (Mus/Bac) or saline into the IC (N = 8). Mean ± SEM. The y axis represents the response rate. B Time course of head entry response during Mus/Bac injection day or saline injection day (N = 8). Each dot represents the total number of head entries for each 1-s-long bin. The cue period is shown with a gray shadow. Mus/Bac (●) and saline (▲) are shown. The x axis represents the time from cue, and the y axis represents counted head entries. C. Head entry responses during cue and no-cue periods after injection of Mus/Bac or saline into the somatosensory cortex (N = 8). Responding is shown as a rate in a minute. D. Time course of head entry activity after injection of Mus/Bac or saline into the somatosensory cortex (N = 8). Each dot represents the total count of head entries during one session. Conventions are as in B. Mean ± SEM. ****, $p < 10$ E -5; n.s., not signficant.

agonist baclofen. A mixture of muscimol and baclofen was infused locally and bilaterally in the IC before starting the session. In inactivation sessions, we observed a significant decrease in the number of head entries during the cue period compared with a control saline injection session (Figure 4.3A; repeated measures one-way ANOVA, $F(3,21) = 54.64$, $p < 10$ E -5 with *post hoc* Bonferroni's multiple comparisons test between drug cue and saline cue). Figure 4.3B shows the average time course of head entries before and during the cue. On the day of drug injection, mice showed very little head entry response throughout the cue presentation period compared with the saline session (Figure 4.3B; repeated measures two-way ANOVA, $F(1,14) = 56.32$, $p < 10$ E -5; $p < 10$ E -5 for 2–10 s by *post hoc* Bonferroni's multiple comparisons test). One of the potential disadvantages of local drug injection is the possibility of drug diffusion beyond the site of injection. To confirm that the effect we observed in Figure 4.3A and B is due to specific inactivation of the IC, we performed a new series of experiments where the same volume of drug mixture was injected in the adjacent somatosensory cortex. Statistical comparison of head entry activity during the cue period between the control (saline session) and the drug (muscimol/baclofen session) did not reveal significant differences (Figure 4.3C; repeated measures one-way ANOVA, $F(3,21) = 33.34$, $p > 0.05$; cue-control vs. cue-drug: paired-samples t-test, $p > 0.05$). There was also no significant difference in the time course of the port entry behavior (Figure 4.3D; repeated measures two-way ANOVA, $F(1,14)= 0.9189$, $p > 0.05$). Thus, these results indicate that the inhibition of port entry activity during the cue period was caused by silencing neuronal activity in the IC.

4.2.4. Temporal Inactivation of IC Decreases Port Entry Activity During Cue Presentation Period

To determine whether the role of the IC in mediating conditioned responses was related to the cue-triggered activity, a series of experiments was performed using optogenetic inactivation of the IC, exclusively during the cue presentation. Insular neurons were infected by adeno-associated virus (AAV) carrying either halorhodopsin (eNpHR3.0) with enhanced yellow fluorescent protein (EYFP) or EYFP alone. First, we determined the effectiveness of green laser light (wave length = 532 nm) in silencing transfected IC neurons. Whole-cell patch-clamp recordings in slices showed that neurons in the IC were reliably hyperpolarized by a 1-s-long constant pulse of green light at 10 mW (Figure 4.4A, Top). A similar result was obtained with cell-attached recordings in slices (Figure 4.4A, Bottom). Consistent with this result, extracellular recordings in anesthetized rodents demonstrated a reliable suppression of ongoing firing by 3 s of constant photostimulation at 10 mW (Figure 4.4B). Consistent with other reports (Goshen et al. 2011; Madisen et al. 2012), a small increase in firing was occasionally observed at the offset of photostimulation; however, it was short-lasting. Optic fibers were chronically implanted in the IC of transfected mice to manipulate neuronal activity selectively during the cue presentation period (Figure 4.4C and D). Figure 4.4C and D shows the viral injection sites and the histological verification of positions of optic fibers. Inactivation of the IC in behaving mice relied on a constant single pulse of 11 s at 10 mW. Quantification of the port entry activity during the cue revealed a significant decrease in the halorhodopsin group compared with control animals (Figure 4.5A; halorhodopsin: 71.13 ± 5.031, control: 97.32 ± 4.066, $N = 8$ for each group; independent-samples t-test, $p < 0.01$). No significant difference was observed in the no-

cue period (Figure 4.5B; halorhodopsin: 88.91 ± 22.81, control: 142.2 ± 20.69, $N = 8$ for each group; independent-samples t-test, $p > 0.05$). In addition, no significant difference in port entry activity was observed for 1 s (paired-samples t-test, $p > 0.05$) or 10 s (paired-samples t-test, $p > 0.05$) after the offset of the light (Figure 4.5C; 12–25 s). This result suggests that rebound activity has no influence on conditioned responses. Comparison of the time course of port entry activity between the prestimulation session and laser stimulation session in halorhodopsin-expressing animals revealed a significant effect of laser stimulation (Figure 4.5*C*; repeated measures of two-way ANOVA, $F(1,14) = 13.21$, $p < 0.01$). No effect was observed for laser stimulation in control animals (Figure 4.5D; repeated measures of two-way ANOVA, $F(1,14) = 9.556$ E -005, $p > 0.05$). Altogether, these data suggest that neuronal activity in the IC triggered by a reward-predicting cue plays an important role in modulating approach behavior to a food reward.

4.3. Discussion

The experiments presented here demonstrate that IC activity in mice is significantly modulated by cues for food anticipation. Furthermore, this study shows that silencing the IC during the cue interferes with food-directed behavior. To investigate the role of the IC in mediating cue-triggered behaviors, we used a Pavlovian conditioning paradigm in which a sensory cue (light and tone) was associated with the delivery of a food pellet. Mice reliably learned the association between the cue and food, as evidenced by the consistent observation of food port entries. Electrophysiological recordings of single units in the IC of mice involved in the paradigm revealed a significant contingent of neurons that were modulated by different phases of the task. Neurons changed their firing rates in response to cues, food delivery, and food consumption. Responses to food delivery and food consumption were expected, because they are consistent with the role in taste processing that is traditionally assigned to the IC in rodents. Indeed, the rodent IC is generally considered to be the site of the primary gustatory cortex across species (Jezzini et al. 2013; Yamamoto 1984; Soares et al. 2007; Mackey et al. 1986). Although evidence for responses to anticipatory cues is less in line with classical reports on the function of the IC in rodents, the evidence is entirely consistent with more recent evidence suggesting the involvement of the IC in processing the anticipation of food and reward in general (Balleine & Dickinson 2000; Contreras et al. 2007; Hollander et al. 2008). Analysis of single-unit activity in rats has confirmed the importance of anticipatory cues in shaping neuronal firing. Neurons in the IC can be either excited or inhibited by an auditory cue predicting the availability of tasting solutions (Samuelsen et al. 2012; Gardner & Fontanini 2014). In addition, cues quench the variability of neural responses (Samuelsen et al. 2012). Our results confirm and extend this evidence to mice engaged in a Pavlovian task. A difference between this and prior studies in rodents is the reliance of our experiments on longer lasting cues. The use of a 10-s-long cue allowed for an accurate and robust assessment of conditioned responses. However, the use of longer cues is likely to have an impact on the dynamics of cue responses. Indeed, in these experiments, cue responses appeared to be smaller than the strong, phasic responses seen in previous reports relying on shorter cues (Samuelsen et al. 2012). The presence of cue-related activity in the IC begs the question of its functional role. Can this type of activity, known to be integrated with taste coding (Samuelsen et al. 2012; Gardner & Fontanini 2014), reflect a role of

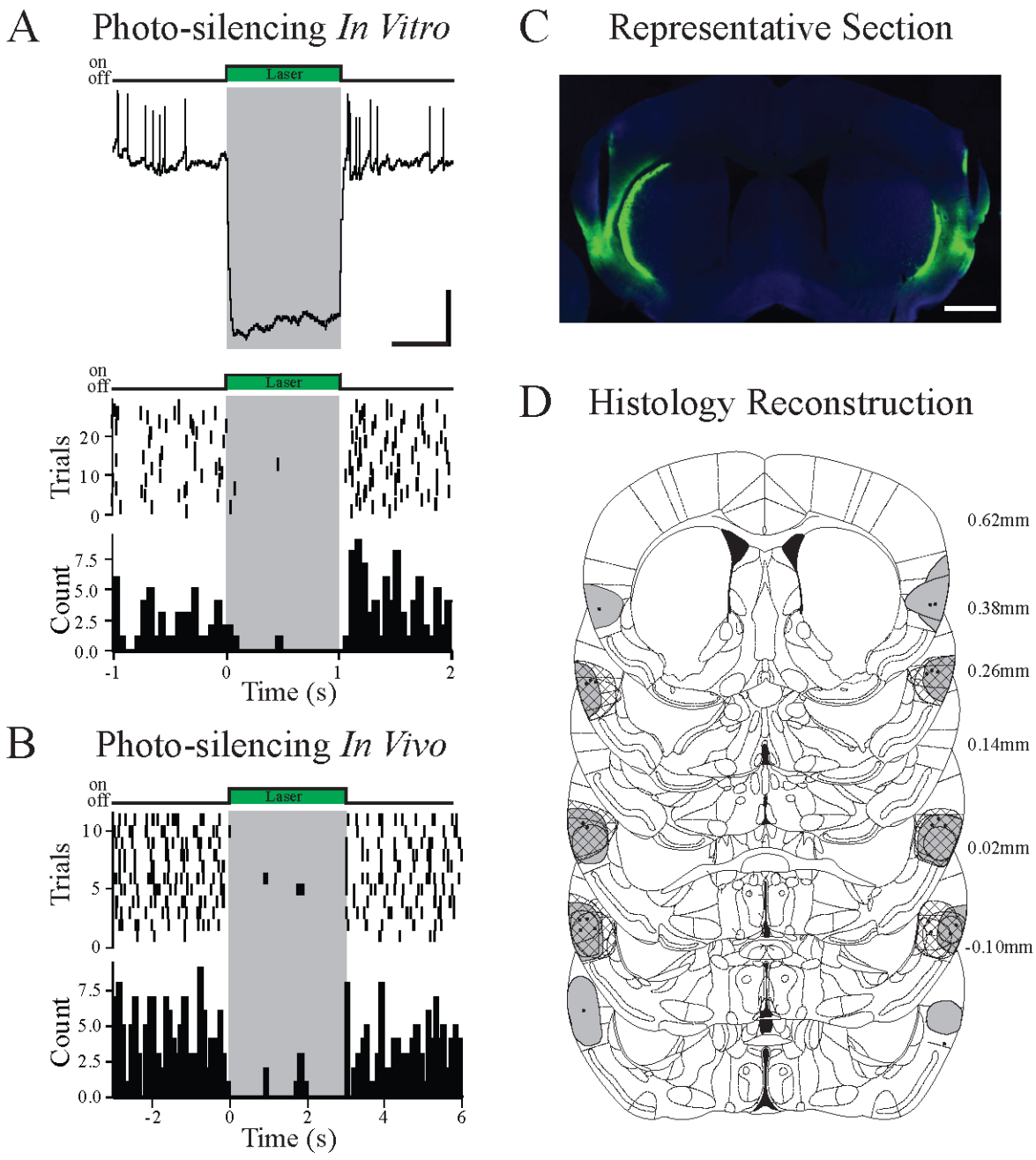
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Figure 4.4: Optogenetic silencing in the IC. A. Effectiveness of photostimulation in slices of the IC. Top: In vitro whole-cell, current-clamp recording showing the average hyperpolarizing response to photostimulation in a halorhodopsin-expressing neuron. Scale bar: vertical, 10 mV; horizontal, 0.5 s. Bottom: Raster plot and PSTH from an *in vitro* cell-attached recording showing silencing of spontaneous spiking as recorded in a cell-attached mode. The x axis represents time, and the y axis represents trials for the raster plot and counts for the PSTH. Gray shading and the green box illustrate the timing of laser stimulation. Photostimulation was performed with a 1-s-long single pulse of green light at 10 mW. B. Raster plot and PSTH showing spiking responses recorded from an IC neuron *in vivo* froman anesthetized mouse. Gray shading and the green box illustrate the timing of laser stimulation. Photostimulation was performed with a 3-s-long single pulse of green light at 10 mW. C. Representative section showing viral injection sites and fiber tracks in ICs. Scale bar: 1 mm. D. Reconstruction of placements of viral injection sites and fiber tips (dots). Expression of GFP fluorescence was drawn either as a gray round shape (halorhodopsin) or a round shape filled with diamond (◊, control).

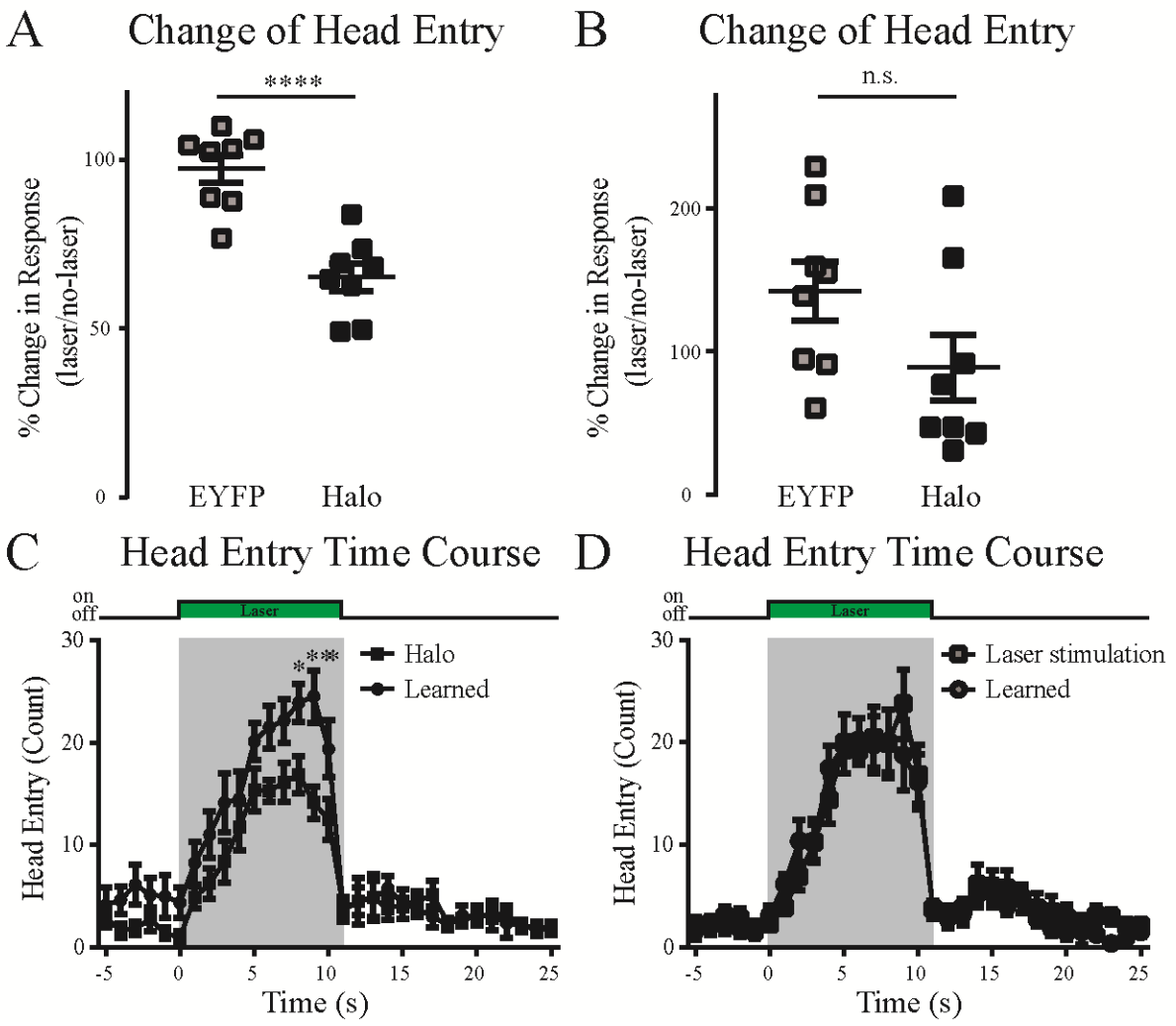


Figure 4.5: Behavioral effects of optogenetic inactivation of the IC during the cue period. A. Comparison of head entry activity during the cue between halorhodopsin (Halo)-expressing animals and control animals (EYFP). Each data point represents the ratio of head entry activity between sessions with laser stimulation and sessions with no laser stimulation during the cue. Control animals (gray square; N = 8) and Halo animals (filled square; N = 8) are shown. The y axis represents the ratio of head entries. B. Head entry activity during ITI. Conventions are as in A. C. Time course of head entry activity during the pre-presentation, cue presentation, and post-presentation periods in animals that have Halo expression in the IC. Animals were well trained to show stable port entry activity (learned), and results were compared with data from sessions with laser stimulation during the cue (N = 8). Performance without manipulation (●) and with laser stimulation (■) are shown. Gray shading illustrates the period of cue presentation. The x axis represents time around cue presentation, and the y axis represents head entries. D. Head entry activity of control animals. Those animals received control virus injection and have only EYFP

expression in the IC. Conventions are as in C. **, $p < 0.01$; ****, $p < 10$ E -5; n.s., not signficant.

the IC in guiding behavior on the basis of expected outcomes? The IC is a highly interconnected area, and its connections to the ventral striatum and to prefrontal cortices (Wright & Groenewegen 1996; Allen et al. 1991a; Kelley 2004) make it an ideal candidate to modulate behavioral output directly.

Classically, the function of the IC has been investigated by relying on permanent lesions or reversible pharmacological manipulations (Braun et al. 1972; Balleine & Dickinson 2000; Contreras et al. 2007; Forget et al. 2010). Inactivation of the IC interferes with an animal's ability to retrieve expected outcomes and perform food-directed behaviors when outcomes are devaluated (Parkes & Balleine 2013). Such a functional role of the IC in flexibly guiding behavior was further supported, and extended to nondietary rewards, by experiments showing that inhibition of orexinergic transmission in the IC reduces nicotine self-administration (Hollander et al. 2008). Lesions and inactivation studies have played a fundamental role in suggesting the importance of the IC in reward-oriented behaviors. However, due to intrinsic limitations of these techniques, it has been difficult to relate the outcome of these experiments to the role of the IC in encoding anticipatory cues. To bypass this technical limitation and directly investigate the behavioral role of cue-related activity in the IC, we took advantage of optogenetics and silenced the IC exclusively during the presentation of the cue. Such a temporally and spatially restricted manipulation resulted in a significant reduction of the conditioned response, providing the first direct evidence, to our knowledge, that IC cue-related activity is necessary for the full expression of conditioned responses to the food-anticipating cue. The behavioral effects observed with optogenetic silencing were smaller than those behavioral effects seen with pharmacological inactivation, likely a consequence of its temporal and spatial selectivity compared with drug infusions. A difference between this and previous studies investigating the role of the IC in mediating associations between cues and outcomes lies in the behavioral task. Whereas previous studies relied on instrumental conditioning, requiring rodents to make associations between action and outcome, the experiments presented here are evaluating stimulus-outcome associations. In the context of instrumentally learned food-oriented actions, lesions and inactivation of the IC have an impact on performance only when animals are required to encode changes in the value of the outcome (i.e., outcome devaluation). When the motivational value of the cue is stable, lesions of the IC have little impact on instrumentally conditioned responses toward food (Balleine & Dickinson 2000). On the contrary, in the case of classical stimulus-response conditioning, optogenetic silencing during the cue disrupts conditioned responses even in motivationally stable contexts. This task-dependent difference in the effects of IC inactivation might reflect a differential involvement of this area in the two behavioral paradigms. Our results predict a strong role of the IC in expressing classical conditioning.

How do the results presented here relate to the large body of literature on the function of the human IC? Our data are entirely consistent with imaging studies, reporting the IC as one of the brain areas that respond to food and reward-predicting cues (Tang et al. 2012). The human IC has also been studied for its role in processing interoceptive signals related to hunger, satiety (Tataranni et al. 1999), disgust (Wicker et al. 2003), cravings (Pelchat et al. 2004), and

homeostasis in general (Craig 2002). Although more experiments will be needed to understand the significance of our data for each of these processes, the results presented here suggest a profound reconceptualization of the IC. Indeed, the IC should no longer be considered just as a sensor of external and internal information. Instead, it should be viewed as a key driver of behaviors based on motivation and retrieval of learned responses. As such, the IC might represent a previously unidentified target for shaping and adjusting maladaptive behaviors.

Chapter 5

General Discussion

Although the gustatory thalamus, i.e., VPMpc, occupies a crucial position in the gustatory pathways, our understanding of its role in taste processing is limited to studies mostly from anesthetized recordings. VPMpc is classically considered a passive relay station of sensory information for GC. However, recent studies call for a revision of the existing view and encourage a systematic investigation on the role of VPMpc in behaving animals. Based on studies on GC (Samuelsen et al. 2012; Gardner & Fontanini 2014), the primary recipient of VPMpc information, it is reasonable to hypothesize that VPMpc may encode taste with complex temporal dynamics and may be involved in encoding taste expectation. Recent studies from other sensory thalamic nuclei (Casagrande et al. 2005; McAlonan et al. 2008; O'Connor et al. 2002; Pais-Vieira et al. 2013; Royal et al. 2006; Ling et al. 2015) provide further support to this hypothesis. My dissertation work aimed to address this hypothesis and provided important updates on the information processing in VPMpc.

5.1. Taste Coding in VPMpc is Temporally Dynamic

Previous electrophysiological studies on VPMpc have greatly contributed to our understanding on taste coding in the thalamus. VPMpc encodes orosensory (tactile, thermal) and chemosensory information (Emmers 1966; Raimond Emmers 1966; Scott & Erickson 1971; Ganchrow & Erickson 1972; Scott & Yalowitz 1978; Nomura & Ogawa 1985; Ogawa & Nomura 1988; Pritchard et al. 1989; Verhagen et al. 2003; Verhagen & Scott 2004). Thalamic neurons are multimodal, i.e., they respond to multiple stimulus properties. Although studies on cortical and limbic processing of taste have emphasized the importance of temporal dynamics, (Katz et al. 2001; Li et al. 2013; Fontanini et al. 2009; Sadacca et al. 2012; Jones et al. 2006), previous research on VPMpc has neglected this aspect (Nomura & Ogawa 1985; Scott & Erickson 1971; Scott & Yalowitz 1978; Pritchard et al. 1989; Verhagen et al. 2003).

In my dissertation study, we analyzed the temporal evolution of response patterns to understand how information is encoded over the course of responses in the VPMpc of alert animals. First, we applied change point analysis (see 6.2.2) on taste-evoked responses to identify periods that showed firing rates modulations (i.e., increases or decreases) significantly different from baseline (Figure 2.2). We found that responses of VPMpc neurons were quite diverse. More than half of taste responses contained multiple modulated periods. Modulation onsets were distributed along the entire analysis period (2.5 s); and modulation durations could be long and short (Figure 2.2C). Applying principal component analysis (PCA), we identified three dominant

response pattern: neural responses could be slow but long-lasting, biphasic or tri-phasic responses (Figure 2.2D).

To understand how taste information could be encoded over time, we applied moving-window analyses on the group of VPMpc neurons that showed taste specificity (i.e., neurons showed different responses to different taste stimuli). To quantify the degree of taste quality information encoded in VPMpc over time, we used two complementary methods. The first one calculated, at each time window, the percentage of neurons that showed significant taste specificity within the taste-specific population. Higher percentage suggested more taste quality information. The second method calculated the population decoding performance based on the cross-validation procedure of a classification method. This procedure divided data into testing trials and training templates, and compared each trial with its corresponding template. The ratio of successfully classified trials to the total trials was defined as decoding performance (see 6.2.4). Higher performance meant better taste coding. Both analyses revealed that the information about taste quality started to appear 100 ms following stimulus delivery and peaked around half second. Significant information about taste was still present at the end of the analysis period (2.5 s), suggesting persistent taste coding in the thalamus. In comparison with the reported time course of taste coding in GC (peak ~ 1 s) (Samuelsen et al. 2013; Piette et al. 2012; Sadacca et al. 2012; Katz et al. 2001; Jezzini et al. 2013), taste quality coding in VPMpc peaked much earlier. This result is consistent with a VPMpc-GC flow of information for taste quality. Indeed, this hypothesis is supported by a recently study, in which the inactivation of VPMpc abolished most taste responses in GC (Samuelsen et al. 2013).

Neglected in previous studies, our analyses revealed palatability coding in VPMpc. We identified palatability-coding neurons using two convergent methods, which have been validated for GC, hypothalamus, amygdala and medial prefrontal cortex (Fontanini et al. 2009; Piette et al. 2012; Sadacca et al. 2012; Jezzini et al. 2013; Li et al. 2013). The first method was based on a within-neuron analysis of response similarity, called palatability index (PI) (see 6.2.6). This method identified neurons responding similarly to tastants with similar palatability (e.g., S and N) and differently to tastants with opposite palatability (e.g., S and Q). The second method relied on the correlation between taste responses and taste hedonic rank (i.e., S>N>C>Q). Both methods yielded a similar amount of VPMpc neurons encoding palatability.

The time course of PI revealed that the palatability information in VPMpc appeared around 0.2 s and peaked around 0.5 s. Compared with the reported time course of palatability coding in GC (Katz et al. 2001; Jones et al. 2006; Piette et al. 2012; Sadacca et al. 2012; Jezzini et al. 2013), palatability information appears earlier in VPMpc. Since PBN is upstream to VPMpc in taste processing (Bester et al. 1999; Ogawa et al. 1987; Krout & Loewy 2000; Karimnamazi & Travers 1998; Norgren & Lundy 1995) and has been indicated in palatability coding (Yamamoto et al. 1994), it might provide taste palatability information to VPMpc. Interestingly, we noticed that the time course of VPMpc palatability coding had an earlier and a late component (Figure 2.4). Single cell analyses also revealed that some neurons showed palatability coding in early period, and some in late. The late component appeared after the peak time of palatability coding in GC. It suggests that the late component might be due to feedback projections from GC.

Our results are consistent with a transfer of information across the PBN-VPMpc-GC axis. While the early onset of taste quality and palatability coding in VPMpc is likely due to a rapid inflow of information coming from PBN, the persistent taste coding and the reverberation-like palatability coding may be the result of recurrent interactions of VPMpc with PBN and GC (Yamamoto et al. 1980; Jones et al. 2006; Sherman 2012). This speculation is supported by the reciprocal connections between the gustatory thalamus, PBN and GC (Allen et al. 1991b; Shi & Cassell 1998; Holtz et al. 2014).

5.2. Effects of Expectation on Taste Coding in VPMpc

Neural responses in sensory thalamus differ depending on the state of the animal. Anesthesia levels influence responses in the visual thalamus, i.e., lateral genicular nucleus (LGN) (Weyand et al. 2001). Sleep and wakefulness have also shown great impacts on thalamic responses to sensory stimuli. During wakefulness, alertness affects thalamic responses and information coding in LGN (Swadlow & Weyand 1985; Li et al. 1999; Bezdudnaya et al. 2006; Cano et al. 2006). Responses to sensory stimuli in the ventral posteromedial nucleus of the thalamus (VPM) have also been found to differ across quiescence, whisker twitching, and exploratory whisking (Fanselow & Nicolelis 1999). Moreover, neural responses in LGN are modulated by spatial attention (O'Connor et al. 2002; McAlonan et al. 2008) and reward (Casagrande et al. 2005). These evidence suggest that thalamic coding is dynamic and changes according to the behavioral demand of the animal (Nicolelis & Fanselow 2002b). Although no study has investigated how taste coding in VPMpc is affected by the behavioral state of the animal, it is reasonable to expect that VPMpc is also under the modulation of the state of the animal.

In my dissertation study, I investigated the effects of general expectation on taste coding in VPMpc, by comparing responses to self-administered and cued tastants with those evoked by passive and un-cued deliveries. We found that taste expectation improved taste quality coding at both single neuron and population levels. Compared with the unexpected condition, taste-evoked responses in the expected condition became more discriminable across tastants, resulting in an increase of decoding performance.

Analysis of neural responses revealed that the number of neurons that coded for taste was no different between the two conditions. However, when taste deliveries were expected, more neurons encoded multiple tastants and less neurons encoded just a single tastant, compared with the unexpected condition (Figure 2.5). This result indicated a shift from relative sparse to dense coding. This is consistent with the hypothesis that the coding scheme adopted is dynamic and dependent on animals' behavior (Barak et al. 2013). In our study, dense coding increased the coding performance.

Besides improving taste coding, general taste expectation accelerates coding in VPMpc. The time course analyses of taste coding showed that, while passively delivered tastants appear not to be coded very effectively in the first 200 ms following stimulus onset, self-administered tastants are encoded as early as in the first 100 ms (Figure 2.3 & 2.7). In addition, the onsets of

taste coding of single neurons were earlier in the expected condition relative to the unexpected condition (Figure 2.7). This result dovetails nicely with similar observations in GC (Samuelsen et al. 2012), implying a close relationship between these two regions. In principle, this acceleration of taste coding may provide animals the advantage to decide faster whether to ingest or reject a taste solution.

5.3. Cue Responses in VPMpc Encode Taste Anticipation

Evidence from human fMRI studies has revealed that sensory thalamus can participate in the processing of anticipatory signals. When human subjects directed attention to the visual periphery to expect visual stimuli, BLOD signals in LGN increased (O'Connor et al. 2002). Previous studies on VPMpc suggest the potential involvement of VPMpc in processing taste anticipation. VPMpc lesions impair taste anticipatory behaviors (Reilly & Pritchard 1997; Reilly 1998; Reilly & Trifunovic 1999a; Schroy et al. 2005). Anticipation-like neural modulations prior to liquid deliveries have been reported in VPMpc of alert monkeys (Pritchard et al. 1989). Moreover, inactivation of VPMpc changed the temporal pattern of the responses to taste predicting cues in GC (Samuelsen et al. unpublished results), suggesting that VPMpc neurons may show cue-related modulation.

The work in my dissertation show that cues predicting the availability of taste activate VPMpc neurons. To demonstrate that cue responses were specific to the predicted value of the tone, a second tone was played, which differed from the cue by its frequency. The second tone was not reinforced so that it did not gain any associative value and was called pseudo cue. We found that only a small percentage of neurons in VPMpc showed modulations triggered by the pseudo cue (Figure 3.1). This result suggests that cue responses are driven by the predictive value of the tone, not by its sensory identity. We also excluded potential confounding factors from motor movements, by recording EMG signals from the anterior digastric muscle (a jaw opening muscle), simultaneously with neural recordings in VPMpc (Figure 3.2). Our analyses showed that even the earliest mouth movement occurred later than the cue response onset. This result demonstrated that cue responses in VPMpc were not secondary to motor movements either. Altogether, this evidence suggests that cue responses in VPMpc encode taste expectation.

Additional analyses of our data show that cue responses in VPMpc are state-dependent and predict behavioral outcomes. By relating cue-evoked activity with animal behaviors, we found that the strength of cue responses in VPMpc correlated with the behavioral reaction to the cue. Cues followed by a behavioral response (the "Respond" trials) evoked stronger modulations than cues not associated with any response (the "Ignore" trials) (Figure 3.3). The two trial types also showed differences in animal behaviors prior to cue onsets. While rats waited by the nose port in the Respond trials, they seemed to lose interest in the cue in the Ignore trials. Analyzing local field potentials prior to the onset of the cue revealed that the power of α oscillation was higher in the Ignore trials compared with that in the Respond trials. The power of α oscillation inversely correlates with the levels of attention. High α power is associated with inattention and low α power is associated with attention (Haegens, Nácher, et al. 2011; Haegens, Händel, et al. 2011; Haegens et al. 2012; Bastos et al. 2014). Thus, when the animal was attentive, as indicated

by a low power in α band, the cue-evoked response was larger and rats responded correctly (Figure 3.3). These results suggest that the state of the animal, i.e., attentiveness, modulates neural responses to cues and leads to distinct behavioral outcomes. Our findings are consistent with the hypothesis that neural coding is adjusted according to the animals' behavioral demand (Nicolelis & Fanselow 2002b), and identify thalamus as an important place for this modulation (Saalmann & Kastner 2011; Sherman 2006).

5.4. Sources of Anticipatory Activity

The work in my dissertation on thalamic processing reveals that taste coding in VPMpc is modulated by expectation and VPMpc encodes taste anticipation by responding to taste cues. These findings suggest an important role of VPMpc in processing higher-order functions, such as taste expectation. These phenomena are very similar to recent findings in GC: taste coding in GC is also improved by expectation (Yoshida & Katz 2011; Samuelsen et al. 2012); GC neurons respond to both general (Samuelsen et al. 2012) and specific taste cues (Gardner & Fontanini 2014). Considering the massive projection from GC to VPMpc (Holtz et al. 2014), I speculate that GC may be a major contributor to the modulations we observed in VPMpc. Indeed, studies on other corticothalamic (CT) pathways have shown powerful modulations on thalamic neurons. Stimulations of the cortex cause complex modulations of activity in thalamic neurons, including: facilitation, suppression, and changes of firing pattern (Crandall et al. 2015; Briggs & Usrey 2008; Sillito et al. 2006; Alitto & Usrey 2003; He et al. 2002; Sillito & Jones 2002; Fanselow et al. 2001; Villa et al. 1991). Cortex inactivation also abolishes whisker twitching behavior mediated by the CT pathway (Fanselow et al. 2001; Nicolelis & Fanselow 2002a). Mechanistic studies have shown that CT glutamatergic projections can either depolarize thalamic neurons directly, or indirectly inhibit them via inhibitory interneurons in the thalamic reticular nucleus (Crandall et al. 2015; Hayama et al. 1994). These synaptic inputs can control the resting membrane potential of thalamic neurons, which governs the state of T-type Ca^{2+} channel, and switch thalamic neurons between firing single spikes (tonic mode) and bursting (burst mode) (Sherman 2001; Llinás & Steriade 2006). Recent experiments have further revealed that the CT pathway can either facilitate or suppress thalamic activity depending on the frequency it is activated (Crandall et al. 2015). Therefore, the CT pathway has the capacity to modulate VPMpc taste responses and drive cue responses.

In addition, sensory thalamic nuclei, including VPMpc, receive innervations from neuromodulatory systems, such as cholinergic and monoaminergic centers (Steriade et al. 1990; Steriade et al. 1988; Hallanger et al. 1987). Similar to CT inputs, neuromodulatory afferents can modulate the resting membrane potential of thalamic neurons and control their firing mode (Devilbiss & Waterhouse 2004; Steriade et al. 1991; Dossi et al. 1991; Berridge & Waterhouse 2003; Nadim & Bucher 2014). Neuromodulatory nuclei centers have been shown to be involved in modulating the behavioral state of the animal (Berridge & Waterhouse 2003; Aston-Jones & Cohen 2005; Lee & Dan 2012). For instance, firing activity in the locus coeruleus (LC), the major source of noradrenergic inputs, correlates with behavioral performance (Usher 1999; Aston-Jones & Cohen 2005). This phenomenon is similar to the state dependent modulation of cue responses observed in VPMpc (Figure 3.3), suggesting a potential relationship between the

two modulations. Thus, neuromodulatory innervations in VPMpc can contribute to state-dependent modulations in VPMpc.

In addition to corticothalamic inputs and neuromodulatory inputs, afferents from the thalamic reticular nucleus (TRN) can mediate complex modulations in VPMpc. Different TRN sections receive projections from sensory cortices, limbic areas (e.g., amygdala), and prefrontal cortices (Zikopoulos & Barbas 2012; Crabtree et al. 1998). Fast interconnections across different TRN sections (Crabtree et al. 1998; Kimura et al. 2007; Hanamori 2003; Pinault & Deschênes 1998a; Pinault & Deschênes 1998b) via chemical and electrical synapses (Cruikshank et al. 2005) make TRN an ideal highway for information integration and cross-modal modulation. Indeed, previous studies have shown that TRN modulates attention signals in the sensory thalamus (McAlonan et al. 2008; McAlonan et al. 2006; Halassa et al. 2014).

5.5. Significance of Cue-evoked Activity

5.5.1. Behavioral Significance of Cue-evoked Activity in Mouse GC

Cue-related activity has been extensively studied in the limbic system and higher-order regions. Studies have revealed that cue-related activity carries information important for animals' behavior. Numerous studies have shown that artificial activation of cue responsive areas leads to anticipatory behaviors, while disruption of cue responses results in behavioral deficits (Tye et al. 2011; Stuber et al. 2011; Nieh et al. 2015; Jennings et al. 2015; Tsai et al. 2009). Discovery of cue responses in the taste pathway led to the hypotheses that cue-related activity may be involved in representing gustatory signals, i.e., taste/food expectation, and may be important in mediating food/reward seeking behavior. In my dissertation study, we collaborated with colleagues in the group led by Dr. Bonci at NIDA and tested this hypothesis.

Our results demonstrate that mouse GC is important for mediating conditioned responses to food cues. In this study, using Pavlovian conditioning, we first developed a measurement for conditioned responses to food cue. We paired an 11-s-long compound cue (tone + light) with a food pellet delivered at the 10^{th} s in a food port. After several pairing sessions, mice showed elevated food-port entering behavior during the cue period. By performing electrophysiological recordings in alert, task-engaged mice, we found neurons in mouse GC showed cue related modulations. To determine if cue-related activity in GC was important for this conditioned behavior, we first performed drug infusion to inactivate GC activity and then optogenetic inactivation, which only affected GC activity during the cue. Both manipulations impaired the conditioned behavior (Figure 4.3&4.5). Therefore, we established the behavioral significance of cue responses in the taste pathway.

Given the close relationship between GC and reward circuits (Maffei et al. 2012), there are multiple ways for GC to mediate these behavioral effects. GC projects to prefrontal cortices (Allen et al. 1991b), amygdala, lateral hypothalamus, and striatum (Cardinal et al. 2002). All these regions are important in guiding behavior at different steps (Everitt et al. 2003; Cardinal et al. 2002; Belin et al. 2013). For instance, the striatum mediates goal-directed behavior;

corticostriatal projections have been shown to affect decision making (Xiong et al. 2015; Znamenskiy & Zador 2013). Further experiments are required to understand the interaction between GC and these areas in food-guided behaviors.

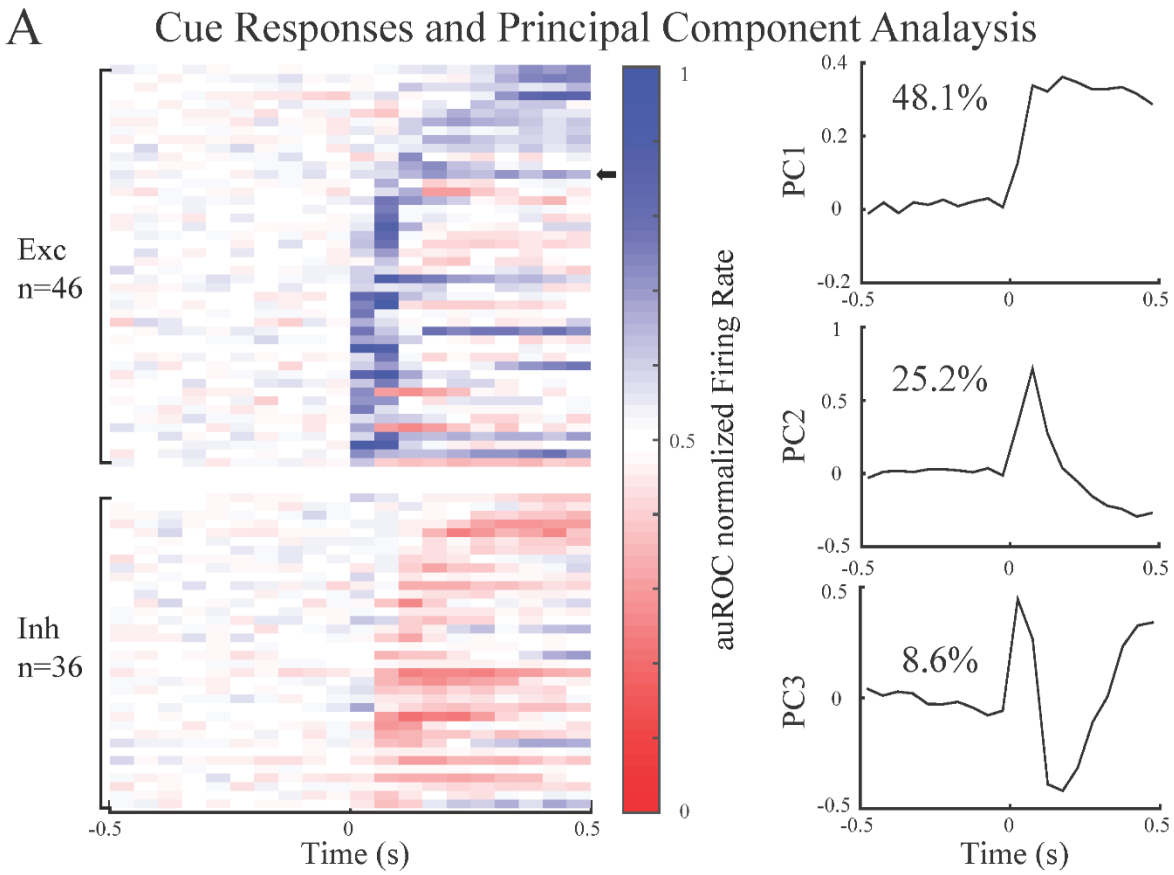
5.5.2. Interpreting Cue-evoked Activity in the Context of Current Theories

Cue-evoked representations are vital for learning and driving behavior. Animals learn the meaning and the predictive value of cues, so that they can adapt their behavior, such as finding food and avoiding danger. Inspired by behavioral studies and learning theory, cue related activity has been widely studied and cue responses have been found in many brain regions, such as VTA, OFC, the striatum, basal forebrain, and so on (Schultz 1997; Schoenbaum et al. 1998; Stalnaker et al. 2012; Lin & Nicolelis 2008; Cohen et al. 2012; Z. Liu et al. 2014; Usher 1999). In this section, I will compare cue responses found in the taste pathway with cue responses in other regions, in order to provide insights for future studies on cue responses in the taste system.

Using a cue-taste association task, my dissertation work and previous studies (Samuelsen et al. 2013; Samuelsen et al. 2012; Gardner & Fontanini 2014) have shown that neurons in the taste pathway, i.e., GC and VPMpc, respond to taste anticipating cues. These studies demonstrate that cue responses are neither simply sensory nor motor related, but represent the cognitive processing triggered by the cue. This cognitive process, which we call taste expectation, is complex and has multiple facets, such as salience (i.e., how important is a stimulus), value (i.e., is it rewarding or aversive) and taste quality (i.e., is it sweet, bitter, salty, or sour). Visual inspection of single-neuron cue responses unveiled a great heterogeneity of cue responses in GC and VPMpc (Figure 5.1A, left). Some responses were transient and phasic, some showed sustained activation, and some others showed multi-phasic response pattern (Figure 5.1A, left). Applying principal component analysis confirmed this intuition; several dominant patterns could be extracted (Figure 5.1A, right). This result suggests that different cognitive variables can be encoded by different response dynamic patterns or different components of the cue responses.

This hypothesis is supported by recent studies in GC. By associating sucrose and quinine with different cues, Gardner et al. found that GC had groups of neurons exhibiting different responses to the two cues. One group responded only to the sucrose-predicting cues; another group only to the quinine-predicting cues; a third group responded to both. Within the third group, some showed distinct, time-varying responses to the two cues, while others responded the same. The example neuron that showed the same responses to the two cues had a phasic response (Gardner & Fontanini 2014). It resembled the cue responses that signal stimulus salience in the basal forebrain (Lin & Nicolelis 2008). Thus, the phasic component of cue responses may signal the salience of cues, despite their different predictive value.

Cue-specific responses represent different expected outcomes (Gardner & Fontanini 2014). However, sucrose and quinine have different reward values (sucrose is rewarding, while quinine is aversive) and different sensory properties (sweet vs. bitter). Whether and how these two separate dimensions are encoded are still unknown. While specific behavioral design will be required to address these questions, previous studies have provided some insights.


A
Cue Responses and Principal Component Analysis
Exc
n=46
Inh
n=36
auROC normalized Firing Rate
Time (s)
PC1
48.1%
PC2
25.2%
PC3
8.6%
Time (s)

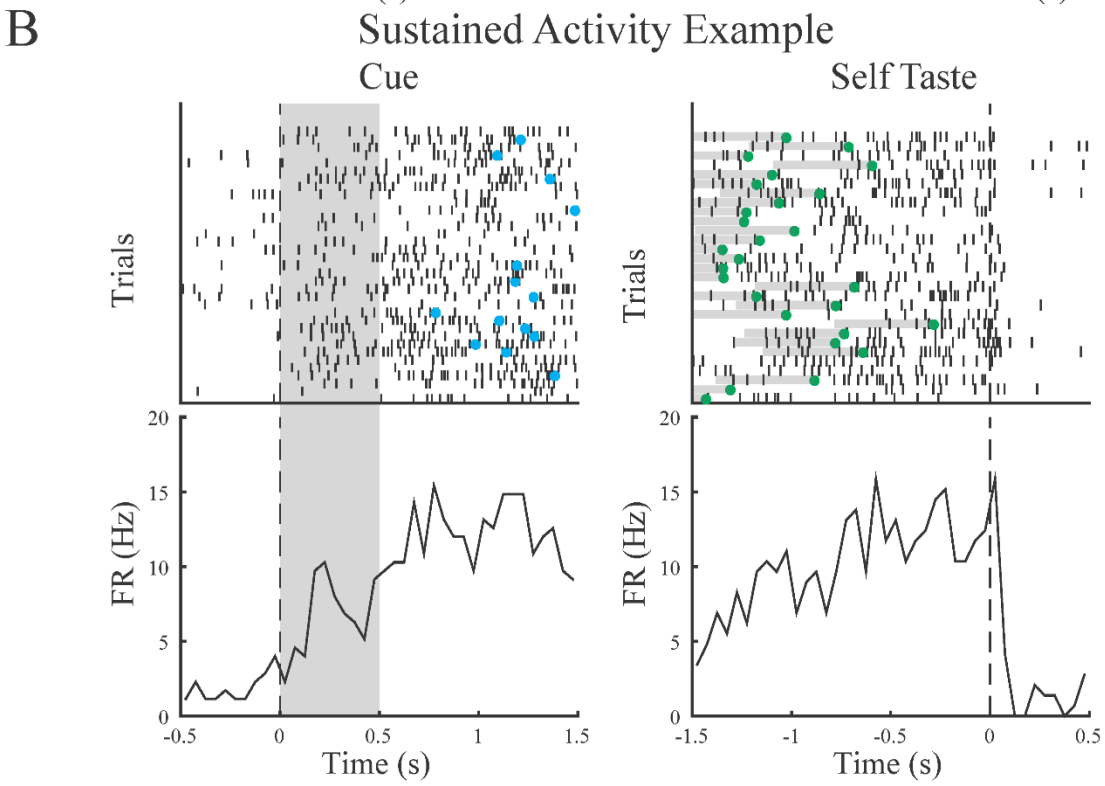

B
Sustained Activity Example
Cue
Self Taste
Trials
FR (Hz)
Time (s)
Trials
FR (Hz)
Time (s)

Figure 5.1: Heterogeneity of VPMpc cue responses. A. Identification of response patterns with principal components analysis (PCA). Left: pseudocolor plot of auROC normalized responses to cue. Y axis indicates neuron number. Neurons are grouped by response type (excitatory or inhibitory) and sorted by response latency. X axis indicates time, which is aligned when the stimulus is delivered (time zero). Arrow indicates the neurons shown in B. Right: eigenvectors shown for the first three principal components. Top: monophasic component accounting for 48.1% of variance; middle: biphasic component accounting for 25.2% of variance; bottom: triphasic component accounting for 8.6% of variance. B. A representative example showing sustained activity during the delay period. Left: activity is aligned to cue onsets. After the termination of the cue, activity stays significant above baseline. Cyan dots indicate self-administration. Shaded box indicates cue presentation. Right: activity is aligned to self-adnimistrations. This neuron is inhibited by taste. Green dots indicate the termination of the cue presentation. Shaded boxes indicate cue presentations.

Different aspects of cues may be encoded by distinct time components in the responses. In a study on cue responses in the non-primary thalamic nucleus (i.e., the posterior thalamic region), Komura et al. found that reward predicting cues elicited responses featuring two components: an early, phasic and a late, sustained (Komura et al. 2001). By manipulating the sensory properties (e.g., tone frequencies) and the reward value (e.g., size of sucrose reward) of cues, the authors showed that while the early component signaled the sensory modality of cues, the late component correlated with reward value (Komura et al. 2001).

Different elements may also be encoded by heterogeneous responses via the mechanism of mixed selectivity. Cue responses in the prefrontal cortex (PFC) are extremely diverse and encode many variables, such as animals' decisions, task rules, and reward (Wallis et al. 2001; Genovesio et al. 2005; Mansouri et al. 2006; Mansouri et al. 2007). A single PFC neuron merely responds to a single event, but shows selectivity to a combination of different aspects of the behavioral task. This phenomenon is called "mixed selectivity". Such mixed selectivity distributes information in a high-dimension space supported by neural population activity. Both theoretical and experimental studies have demonstrated its advantages in neural computation and importance in complex cognitive tasks (Miller & Cohen 2001; Goldman-Rakic 1987; Zipser & Andersen 1988; Pouget & Sejnowski 1997; Pouget & Snyder 2000; Salinas & Abbott 2001; Rigotti et al. 2013; Liu et al. 2014).

One final aspect of cue responsiveness might be related to working memory. The task used in my dissertation work contains a delay period (i.e., a time window between cue offsets and taste self-administrations), where no sensory stimulus is present. Visual inspection suggests that some of the cue-evoked, sustained activation persists after the termination of cue (Figure 5.1B). This feature resembles the signature of working memory in PFC (Romo et al. 1999; Funahashi et al. 1989; Fuster & Alexander 1971). The sustained activity in PFC during the delay period after cues has been shown to be important for memory retention and guide animals' behavior (Liu et al. 2014; Rigotti et al. 2013; Parent et al. 2015). Thus, the cue-evoked, sustained activity in the taste system may encode working memory and guide animals' behavior.

These speculations suggest a series of future investigations to dissect the variables that are encoded by the cue responses in the taste pathway. Future investigations will apply sophisticated behavioral, recording, and analytical techniques to understand the information encoded by cues. In addition, more studies will be needed to investigate the mechanisms for the emergence of cue responses (i.e., circuit and cellular mechanisms). Addressing these issues will provide a fundamental understanding on how sensory areas of alert animals integrate sensory and cognitive signals. The work presented in my dissertation greatly advanced our knowledge of thalamic processing and represents the foundation for future studies.

5.6. Revised Model and Speculations on VPMpc Circuits

My thesis work has shown that VPMpc not only processes the sensory aspects of taste, i.e., taste quality, but also encodes the psychological aspects, i.e., taste palatability. Moreover, VPMpc neurons can encode taste expectation by responding to the cue predicting the availability

of taste. This result challenges the classical view of VPMpc as a sensory relay and suggests an integration of information in the gustatory thalamus. Based on these results, the classical view that hypothesizes functional segregation between VPMpc-GC and BLA-GC pathways needs to be revised. In the thalamocortical (TC) pathway, VPMpc integrates information regarding both sensory and psychological domains of taste experience. These findings open a new direction to study information integration and its potential sources in the VPMpc-related circuit.

VPMpc receives four major projections: glutamatergic projections from the parabrachial nucleus (PBN) in the brain stem, glutamatergic projections from the gustatory cortex (GC), GABAergic projections from the thalamic reticular nucleus (TRN), and inputs from neuromodulatory systems in the brain stem (Figure 5.2). In the following paragraphs, I will discuss my speculations on how information provided by those inputs is integrated and suggest future experiments.

PBN, which sits two synapses away from taste buds, projects to VPMpc and limbic areas in the forebrain. Previous studies have focused on the processing of taste chemical identity in the gustatory system and have shown a hierarchical relationship in the PBN-VPMpc-GC pathway (Verhagen et al. 2003; Carleton et al. 2010). Indeed, in my thesis work, the time course observed on taste identity coding in VPMpc was earlier than that in GC. In addition, my results revealed the coding of taste palatability in VPMpc. The time course of palatability coding in VPMpc was also earlier than that in GC, suggesting a flow of taste palatability from VPMpc to GC. The source of taste palatability, I speculate, may be PBN. Although electrophysiological studies in PBN focused on taste identity coding (Rosen et al. 2011; Weiss et al. 2014), PBN neurons activated by different tastants have been shown to cluster anatomically according to palatability, hence processing taste palatability (Yamamoto et al. 1994). This indicates that PBN can provide the substance for palatability coding in VPMpc. Future investigation should analyze taste responses in PBN regarding palatability.

The most abundant input to VPMpc is the corticothalamic (CT) pathway (i.e., GC-VPMpc) (Maffei et al. 2012; Holtz et al. 2014). CT projections also send collaterals to the thalamic reticular nucleus, which provide inhibitory modulations to VPMpc (Groenewegen & Witter 2004). Thus, the CT pathway can both excite and inhibit VPMpc neurons. Indeed, both effects have been observed in the CT pathways of other sensory systems (Crandall et al. 2015; Briggs & Usrey 2008; Sillito et al. 2006; Alitto & Usrey 2003; He et al. 2002; Sillito & Jones 2002; Fanselow et al. 2001; Villa et al. 1991). As GC has been shown to process both sensory and affective aspects of taste experience (Stapleton et al. 2006; Yoshida & Katz 2011; Piette et al. 2012; Sadacca et al. 2012; Samuelsen et al. 2012; Gardner & Fontanini 2014), information conveyed by the GC-VPMpc pathway can be heterogeneous. Therefore, the CT pathway may modulate VPMpc on the coding of taste identity, palatability and taste expectation.

To understand the modulation from GC onto VPMpc, first, I would suggest dual recordings from GC and VPMpc. Neuron-pair analyses would reveal how neurons between the two areas interact and how information flows. Results from dual recordings would help to develop hypotheses on the roles of GC in VPMpc processing. Second, careful circuit dissections are needed to test those hypotheses. Optogenetic tools should be implemented to investigate the temporal effects of GC inactivation and pathway-specific modulations. As taste coding evolves

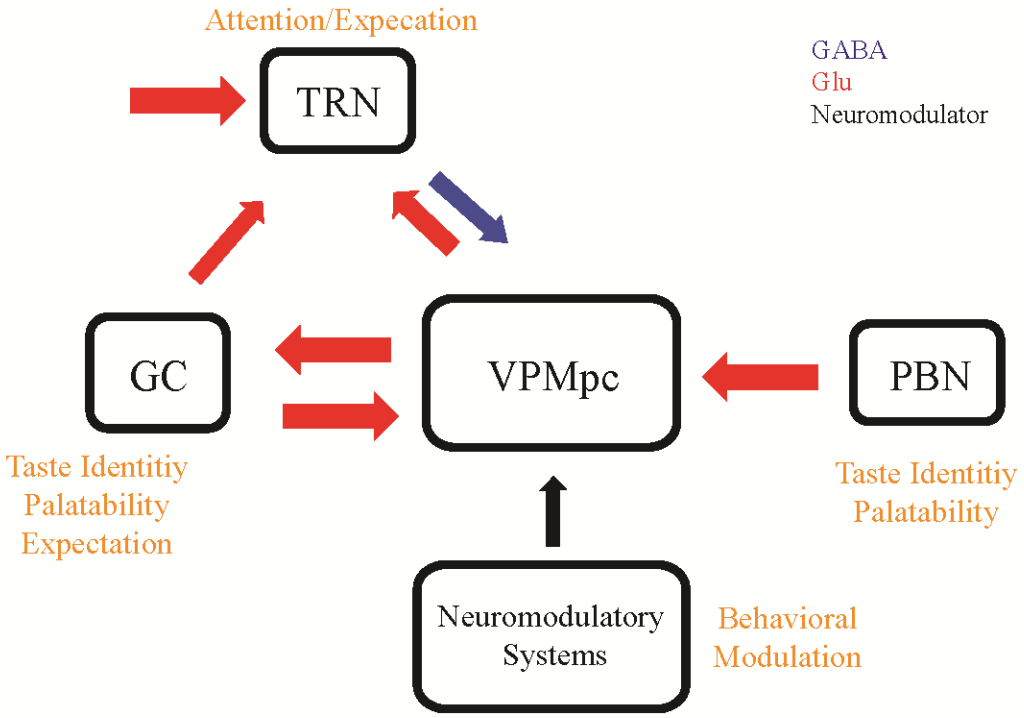


Figure 5.2: Potential Sources for Information Integration in VPMpc-related Circuits. Boxes indicate nuclei. Arrows represent projections: GABAergic projection in blue, glutamatergic projections in red, and neuromodulatory innervations in black. Orange indicates the potential contribution to the information processing in VPMpc. TRN: thalamic reticular nucleus; GC: gustatory cortex; VPMpc: parvicellular portion of the ventral posteriomedial nucleus of thalamus; PBN: parabrachial nucleus; GABA: GABAergic; Glu: glutamatergic.

over time, it would be important to investigate the effects of GC inactivation at different "epochs" of taste processing (Katz et al. 2001). Those experiments will shed light on how GC contributes to the persistent taste identity coding and reverberant palatability coding in VPMpc (Figure 2.3&2.4). As GC encodes taste expectation by cue responses and expectation modulates pre-taste activity (Yoshida & Katz 2011; Samuelsen et al. 2012), it is reasonable to hypothesize that GC also contributes to cue responses and the modulation of expectation on taste responses in VPMpc. GC inactivation can also be used to test these hypotheses. Moreover, optogenetics allows specific manipulation of the CT projections by targeting axon terminals. This approach will help dissect the components carried by CT direct projections and those mediated by TRN.

Comparison of cue responses in GC and VPMpc shows similar latencies. It implies that cue responses in VPMpc may not depend on GC exclusively and suggests other potential sources. I speculate TRN may be one source. Anatomically, TRN forms a thin sheet of neurons and covers the entire rostral and lateral boarder of the dorsal thalamus, which contains sensory thalamic nuclei. TRN can be divided into subsections, which are connected with sensory thalamic nuclei, limbic regions (amygdala, mediodorsal thalamic nucleus), and prefrontal regions (Zikopoulos & Barbas 2012) respectively. Although different sections are spatially segregated, synaptic connections across sections and interactions across different sensory modalities have been observed (Shosaku et al. 1989; Cruikshank et al. 2005; Crabtree & Isaac 2002). Therefore, besides driven by the axon collaterals of TC and CT pathways, TRN provides a circuit for information integration from higher-order regions and for cross-modal modulation. Thus, TRN has been suggested in higher-order modulations, such as attention (Crick 1984; Sherman 2007). Indeed, both recordings (McAlonan et al. 2008; McAlonan et al. 2006) and optogenetic manipulations (Halassa et al. 2011; Halassa et al. 2014) have revealed its roles in attentional and behavioral modulations. I speculate that cue responses could originate in this pathway. To test this hypothesis, recordings from the gustatory section of TRN (Hayama et al. 1994) will be required. Circuit dissection methods will also be needed to establish causal relationship.

Moreover, VPMpc is innervated by projections from the neuromodulatory systems in the brain stem, such as cholinergic neurons in laterodosal nucleus and non-adrenergic neurons in locus coeruleus (LC) (Steriade et al. 1990; Steriade et al. 1988; Hallanger et al. 1987; McCormick 1989). Thalamic neurons can be modulated by these innervations (Devilbiss & Waterhouse 2004; Steriade et al. 1991; Dossi et al. 1991; Berridge & Waterhouse 2003; Nadim & Bucher 2014); and those neuromodulatory centers have been suggested to participate in task performance and the state of the animal such as arousal (Berridge & Waterhouse 2003; Aston-Jones & Cohen 2005; Lee & Dan 2012). In particular, the behavioral modulation of cue responses observed in VPMpc neurons (Figure 3.3) resembles those in locus coeruleus (LC) (Usher 1999; Aston-Jones & Cohen 2005). Thus, I speculate that LC contributes to the modulation of cue responses in VPMpc. Circuit dissection approaches will also be needed to understand this pathway.

Chapter 6

Methods

6.1. Methods and Materials

6.1.1. Experimental Subjects

All the experimental procedures were performed according to federal, state, and university regulations regarding the use of animals in research and approved by the Institutional Animal Care and Use Committee of Stony Brook University. Eleven adult female Long-Evans rats (280~350 g) served as the subjects in Chapter 2 and 3. Forty eight adult C57BL/6J mice were used in the study in Chapter 4. Animals were maintained on a 12 hr light/12 hr dark schedule and were given *ad libitum* access to chow and water, unless otherwise specified.

6.1.2. Electrode Preparation

All recording electrodes were custom-made and assembled in the lab.

Single wire bundles: 25 μm Formvar-coated nichrome wires (A-M systems, Inc., Cat No. 761500) were soldered on to an Omnetics connector (Omnetics Connector Corporation, part No. A79002-001) with a customized electrode interface board (EIB). Electrode bundle with 8 or 16 wires were assembled together with a custom-made micro drive, which allows for ~ 2 mm travel distance. Prior to implantation, electrodes were cut with a sharp scissor and the impedance was brought to ~ 500 kilo-Ω at 1 kilo-Hz. Good separation across electrode was achieved by spreading electrodes with a No.5 forceps (Katz et al. 2001).

Tetrodes: each tetrode consisted of four polyimide-coated nichrome wires (Kanthal Palm Coast; wire diameter 12.7 μm) twisted together using a tetrode-making station (Neuralynx). Four-tetrode bundle/array was assembled together with a custom-made micro drive. Prior to implantation, electrodes were cut with a sharp scissor and gold plated to bring the impedance to ~ 300 kilo-Ω at 1 kilo-Hz (Jaramillo and Zador, 2011). Electrode array: polyimide tubes were arranged into 1x4 or 2x2 arrays with cyanoacrylate glue. Individual tetrodes were inserted into each polyimide tube when assembling micro drives.

EMG electrodes: two Teflon insulated stainless steel (A-M system, Inc., Cat No. 781000) wires were soldered onto Omnetics connector (Omnetics Connector Corporation, part No. A79002-001), and then twisted together to form EMG recording electrode. EMG electrodes were

inserted into PE-10 tubes (A-M Systems, Inc., Cat No.800700), which protected the electrode under the animal's skin. The tip of electrodes was exposed by stripping the insulation. Prior to insertion, a hook was formed, by bending the electrode wires, to help hold electrodes in the muscle.

6.1.3. Surgical Procedures

Rats/mice were anesthetized using an intraperitoneally injected anesthetics cocktails (for rats: ketamine/xylazine/acepromazine [KXA] mixture [in mg/kg respectively: 100, 5.2, and 1]; for mice ketamine/xylazine [KX] mixture [in mg/kg 100 and 10, respectively]) with supplemental doses (30% of induction dose) to maintain surgical levels of anesthesia. After placing animals in a stereotaxic device, the scalp was sterilized with 0.1% iodine and excised to reveal the skull. Holes were drilled for the placement of anchoring screws, recording electrodes, infusion cannulae and optical fibers. Electrodes were inserted dorsal to the targets (Rats: VPMpc [anteroposterior (AP) -3.6 mm, mediolateral (ML) 1.1 mm from bregma, 5.9 mm deep from dura (DV)] (Verhagen et al. 2003; Samuelsen et al. 2013)) in one or both hemispheres. Infusion cannulae for mice (26-gauge; Plastics One) were implanted bilaterally (0.26 mm AP, 3.9 mm ML, at a depth of 3.8 mm relative to bregma). Optical fibers for light stimulation (custom-made implantable optic fiber [4-mm length below ferrule, 200-μm core multimode (Thorlabs), 1.25 mm-outside diameter ceramic zirconia ferrule (Precision Fiber Products)]) were implanted bilaterally (0.26 mm AP, 3.9 mm ML, at a depth of 3.8 mm relative to bregma) in mice, after virus injections.

Virus injection for mice were performed by using a 29-gauge stainless-steel cannula. 0.5 μL of either AAV1-alpha-calcium/calmodulin-dependent protein kinase II promoter (CaMKIIα)-eNpHR3.0-EYFP or AAV1-CaMKIIα-EYFP (National Institute on Drug Abuse Intramural Research Program Optogenetics and Transgenic Technology Core) was infused into the target site at 0.1 μL/min. The injecting cannula was kept in place for at least five minutes before withdrawal.

All implants were cemented to the skull with dental acrylic. Rats were also implanted with intra-oral cannulae (IOC) (Phillips and Norgren, 1970) bilaterally, which were cemented onto the head cap with dental acrylic (Katz et al. 2001; Fontanini & Katz 2005). For a subset of animals, electromyography (EMG) recording electrodes were inserted into the anterior digastric muscle (jaw opener) to monitor mouth movement (Travers et al. 1987; Roitman et al. 2005). EMG wires ran underneath of face skin and came out from the opening on the scalp. EMG connectors were also cemented on the head cap. Animals were given at least one week for recovery. Training started after animals returned to > 80% of their original weight.

6.1.4. Behavioral Training

General expectation behavioral paradigm: after recovery from surgery (at least a week), rats were water restricted (45 min of water/day) and habituated to the behavioral/recording chamber (MED Associate Inc., St. Albans, VT) and to receiving fluids through IOC (Phillips & Norgren 1970; Katz et al. 2001; Fontanini & Katz 2005). Once habituated, rats were trained to self-administer and passively receive tasting solutions. Every trial began with an auditory cue

(3000, 6000, or 9000 Hz, 80 dB, 500 millisecond [ms] long). Rats learned to respond by poking the nose into a port in the chamber after the offset of the cue and within a response window (3 second [s]). Successful responses led to delivery of a taste solution (~ 40 μL delivered by a pressurized, computer-controlled system) into the rat's mouth via a manifold of four polyimide tubes slid into one IOC. Each taste delivery was followed, 5 s later, by a water rinse (~45 μL) delivered into the second IOC. Taste solutions (100 mM NaCl [N], 100 mM sucrose [S], 200 or 100 mM citric acid [C], and 1 mM quinine HCl [Q]) were delivered pseudo-randomly at each trial. We defined this type of trial as self-administration (Self). Upon self-administering the taste, rats were required to wait a variable inter-trial interval (ITI) of 30 to 50 s until the next trial started. At random times during the ITI, taste solutions were delivered by the computer-controlled system in the absence of any cue. These trials were defined as Passive deliveries. Nose poking during ITI was discouraged by adding waiting time (~5 s) every 10 extra nose pokes. After learning the task, rats developed stereotyped behavior: stable responding during response window and very a few spontaneous nose pokes during ITI. Recording sessions normally lasted around 45 min, wherein each taste solution for each condition was delivered in at least 6 trials. For pseudo cue experiments, another pure tone at a different frequency was played during ITI. The pseudo cue did not lead to any reinforcement, thus it was meaningless to animals. After training, rats showed very few nose pokes after pseudo cue. After behavioral sessions, additional water access (~10 min for ~ 10-15 mL) were provided.

Pavlovian condition paradigm: training took place in an operant chamber (ENV-307W; 8.5 inches long × 7.0 inches wide × 5.0 inches high; Med Associates, Inc.) housed within a light-resistant and sound-attenuating cubicle. The chamber was equipped with a food magazine that received 20-mg pellets (5TUL; Test Diet) from a pellet dispenser, and it had a house light and two sound speakers on the wall opposite to the magazine. Computers running the Med-PC-IV program (Med Associates, Inc.) were used to control the chambers and record behavior. After a recovery period from surgery, mice were trained to get food pellets from the food magazine. Fifteen food pellets were delivered randomly over 30 min for 2 d (habituation sessions). On subsequent days, mice underwent Pavlovian conditioning. The combination of tone and house light cues [conditioned stimulus (CS)] was paired with delivery of a 20-mg food pellet [unconditioned stimulus (US)]. The association learning task was adapted from a task described before (Parker 2010). Each training session began with a 45 to 75 s ITI. After the interval period, the CS was turned on for 11 s. A pellet was delivered following 10 s of the CS onset. Mice were allowed to access the food magazine freely, and both the number and timing of head entry to the magazine were recorded. After 11 s of the CS presentation, the ITI was started and the trial was repeated 25 times in each session. Each session lasted for 30 min, and the sessions were continued until the mice showed a stable head entry response. Learning was assessed by comparing anticipatory head entries to the food magazine during the CS and the ITI. Head entry rate was obtained by dividing the number of head entries during each period by time and was converted to rate per minute.

6.1.5. Drug Infusion and Optical Stimulation

For experiments using local drug infusion in GC, mice were injected with 0.3 μL of muscimol/baclofen solution (0.05 μg/μL and 0.02 μg/μL, respectively) or saline at a rate of 0.1 μL/min. Drugs were infused on alternating days through a 33-gauge stainless-steel cannula that

was inserted in the chronic guide cannula and protruded 0.2 mm below the tip. Five minutes after infusion, the injection cannula was removed, and the mice were allowed to recover in their home cage.

For all optogenetic experiments, either eNpHR3.0 with EYFP or EYFP-tagged viral vector under control of the CaMKIIα promoter was used. These viruses provide eNpHR3.0 (or EYFP) expression that is highly specific to pyramidal cells in a cortical structure (Calu et al. 2013). Mice started behavioral training with optical cables connected to the implanted optical fibers on their head cap with a sleeve (Precision Fiber Products). The fiber was connected to a 532-nm laser (OEM Laser) located outside the operant chamber. The laser was controlled with Master-9 (AMPI) and Med-PC-IV. Mice performed the same Pavlovian conditioning task, but the laser was activated for 11 s of each cue presentation period. We used a single laser pulse, which lasted for the entire duration of the cue period. Before testing, the final output of the laser was adjusted to 10 mW. According to the literature (Tye et al. 2011) and prior experience (Chen et al. 2013), this level of output, combined with the characteristics of the fiber used, guarantees a conical spread that can reach a depth of 1 mm from the tip of the fiber.

6.1.6. *In vitro* Electrophysiology

For whole-cell recording, 250-μm coronal slices were cut from virus-injected mice (n = 2). We waited at least 4 wk after virus injection before starting the slice recording experiments. Brains were quickly removed and placed into ice-cold solution bubbled with 95% O_2 and 5% (vol/vol) CO_2 containing the following: 75 mM sucrose, 55 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 10 mM $MgSO_4$, 0.5 mM $CaCl_2$, 26 mM $NaHCO_3$, and 25 mM glucose. After 5 min, brains were blocked and coronal slices were taken. During the recovery period, slices were placed at 35 °C with oxygenated artificial cerebrospinal fluid (aCSF) solution containing the following: 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 1.0 mM $MgCl_2$, 2.4 mM $CaCl_2$, 26 mM $NaHCO_3$, and 11 mM glucose. All recordings were made under continuous perfusion of aCSF at 32 °C with a flow rate of 2 $mL \cdot min^{-1}$. Pipettes (2.5–5 MOhm) for voltage and current-clamp experiments contained the following: 140 mM potassium methylsulfate ($KMeSO_4$), 5 mM KCl, 0.05 mM EGTA, 10 mM HEPES, 2 mM $MgCl_2$, 2 mM ATP, and 0.4 mM GTP (pH 7.2–7.3; 280 mOsm). Recordings were made in the IC from visually identified pyramidal neurons. For recordings of pyramidal neurons, slices were stimulated with 532 nm green light. During recordings, 1 s of light was delivered with a power of 10 mW.

6.1.7. *In vivo* Electrophysiology in Anesthetized Animals

A few animals (N = 3) were used to verify the effects of neuronal inhibition by halorhodopsin. Animals were anesthetized with intraperitoneally injected ketamine and xylazine. The surface of the IC was exposed from the lateral side of the brain. The skull was opened above the IC, and a recording electrode (Neuronexus) was inserted. Stimulation light (532 nm) was delivered through the implanted optic fiber. All recordings were performed with a Blackrock Cerebus system (Blackrock).

6.1.8. *In vivo* Electrophysiological and EMG Recordings in Behaving Animals

A Plexon Multichannel Acquisition Processor (Plexon, Dallas, TX) was used for electrophysiological recordings. Single unit waveforms and local field potentials (LFP) signals were amplified, band-pass filtered (at 300 to 8000 Hz for single units and 3 to 90 Hz for LFP), digitized, and recorded. EMG signals were first amplified using a differential AC amplifier (A-M Systems, Inc., Cat. No. 690000), and fed into the analog signal input channel of Plexon system. Unique single unit waveforms of at least 3:1 signal-to-noise ratio were recorded and isolated relying on voltage threshold detection and a template matching algorithm. Units were further sorted off-line using cluster cutting techniques and examination of inter-spike interval plots (Offline Sorter, Plexon, Inc.; Dallas, TX).

6.1.9. Histology Procedures

At the end of the experiments, rats (or mice) were deeply anesthetized using the KXA (or KA) mixture, and electrolytic lesions (10 μA, 10 s) via selected electrode wires were made to mark final electrode positions. Subsequently, rats were perfused intracardially with 0.9% saline followed by 10% Formalin or with 0.1 M phosphate buffered saline (PBS) followed by 4% paraformaldehyde. Fixed brains were stored in 10% Formalin or PBS and then 30% sucrose solution, and cut into coronal sections with a cryostat (Microm HM 505 E) or a vibratome (VT-1200; Leica) (slice thickness: 40, 50, 80, 100 μm). Standard Nissl staining procedure was used to visualize cannula and electrode positions. Electrode tracks and final positions were verified and reconstructed based on histology, stereotaxic coordinates of initial positioning and recording notes. EYFP expression was verified by fluorescent microscopy; layer structure was visualized by fluorescent Nissl staining (2% Neurotrace [Life Technologies] or 4′,6-diamidino-2-phenylindole).

6.2. Data Analysis

All data analyses were performed using customized MATLAB scripts (MathWorks, Natick, MA). Data are presented as mean ± standard error of the mean (SEM) in the main text, unless otherwise indicated. A bootstrap procedure was used to estimate the mean and 95% confidence interval (CI), and to determine significance; 1000 runs were used, unless otherwise indicated.

6.2.1. Area under the Receiver Operating Characteristic curve (auROC) Method

We used the auROC method for: 1) normalization of firing rate (FR) and quantifying response strength according to its baseline; 2) comparison across taste-evoked responses (see 6.2.5); 3) comparison of state-related modulation (see the 6.2.9). Detailed description of this method can be found in previous publications (Cohen et al. 2012; Jezzini et al. 2013). Briefly, the auROC method compares two distributions of firing rates (or spike counts). It yields a quantity between 0 and 1, which is the auROC value. When comparing taste evoked activity to its baseline using auROC method, values larger than 0.5 indicate that the evoked firing rate is above baseline (i.e., excitatory modulation), whereas values below 0.5 mean that the evoked activity is below baseline (i.e., inhibitory modulation).

6.2.2. Taste Responsiveness (Change Point Method)

Taste responsiveness and response latency were assessed using the previously described adaptive change point method (Gallistel et al. 2004; Jezzini et al. 2013). Briefly, the adaptive change point method relies on the cumulative distribution function (CDF) of spike occurrences across all trials for a given taste for each single unit. One second baseline (i.e., 1 s prior to taste deliveries for Passive taste or 1 s prior to cue onset for Self) and 2.5 s post-taste-delivery period were considered. Only 2.5 s after taste delivery were analyzed for consistency with previous publications on taste processing in alert rats (Katz et al. 2001; Fontanini & Katz 2006; Grossman et al. 2008; Fontanini et al. 2009; Piette et al. 2012; Sadacca et al. 2012; Samuelsen et al. 2012; Li et al. 2013; Samuelsen et al. 2013). Criteria were determined by baseline activity and tolerance level ($p = 0.05$, see (Gallistel et al. 2004)). A significant change in the slope of the CDF was defined as a change point (CP) and indicated the onset of a firing rate modulation. Adjacent modulation periods were compared using paired-sample t-test (based on firing rate during modulated periods) and modulation periods that were not significantly different were merged. Modulation periods were required to last more than 10 ms. *Post hoc* test (paired-samples, one-side t-test, $p < 0.01$) between firing rates in modulated periods and time length matched baseline activity was used to determine significant response periods. A neuron was defined as taste-responsive if it had a significant modulation period for at least one of four taste stimuli. The response latency was determined by the onset of the first response period.

6.2.3. Taste Selectivity

A taste-responsive neuron was considered taste-specific (TasteS) when it featured different responses to different taste stimuli (Jezzini et al. 2013; Samuelsen et al. 2013). Taste evoked activity (from 0 to 2.5 s after taste delivery) was averaged in 250 ms wide bins. A two-way ANOVA (taste identity X time course) was used to determine taste specificity. If the taste main effect or the interaction term was significant ($p < 0.01$) in the above test, a neuron was defined as taste-specific or taste coding.

6.2.4. Taste Classification

To assess how well each taste is encoded by a single unit or an ensemble of neurons, we used a previously described decoding procedure (Jezzini et al. 2013). This analysis is based on a cross-validation procedure and a Euclidean distance-based classifier (see (Jezzini et al. 2013) for detail). In this study, a 250 ms bin size was used. Decoding performance was defined as the ratio of test trials that were classified correctly over all trials. When the decoding performance for a certain taste was larger than chance level (significance was based on a binomial distribution), the unit was deemed as taste coding. For the ensemble-based classification, significant decoding was defined by the lower bound of 95% bootstrapped CI larger than chance level (0.25). The difference between two populations was considered significant when it fell outside of the bootstrapped CI. To estimate the minimal number of neurons needed to reach a decoding accuracy of 95%, we created a large surrogate neuronal population from the available data, by randomly re-assigning the labels of the responses to different tastes (Jezzini et al. 2013; Rigotti et al. 2013). This classification method was also used with a moving window (250 ms bin size, 50 ms step) to assess decoding performance over time.

6.2.5. Taste Palatability

A neuron was defined as palatability-coding, when 1) it was taste-specific, and 2) its responses followed the intrinsic hedonic value of taste. We used two methods to assess palatability coding. Both methods analyzed the entire time course of responses using a sliding window (250 ms bin size, 50 ms step). The first method is based on a palatability index (PI, see 6.2.6). A taste-specific neuron was defined to be palatability-coding, when its PI was significantly larger than the 97.5 percentile of baseline PI distribution for a continuous period of 200 ms (80% of 250 ms window). The second method was based on Spearman's rank correlation between firing rates and hedonic rank of taste (Sadacca et al. 2012). Since the four taste stimuli used in this current study have been widely studied, their preference relationship (i.e., hedonic rank: S > N >> C > Q) has been well established (Harvey J Grill & Norgren 1978; Breslin et al. 1992; Berridge 2000; Sadacca et al. 2012). We calculated the Spearman's rank correlation coefficient (ρ) between firing rates and the taste hedonic rank. When a period longer than 200 ms was significant ($p < 0.05$), the neuron was defined as palatability-coding (this criteria was based on previous studies (Sadacca et al. 2012)).

6.2.6. Palatability index (PI)

To assess palatability-related information, we used a palatability index based on previous work (Grossman et al. 2008; Fontanini et al. 2009; Jezzini et al. 2013). This method considers the difference in evoked activity between taste stimuli with similar and opposite palatability. To avoid biases introduced by differences in firing rates across neurons, we used the auROC method (see 6.2.1) to estimate the difference between taste pairs. Briefly, the auROC method yielded a normalized difference between two firing rate distributions (e.g., between S and N). 0.5 means that two distributions are the same. The absolute deviation from 0.5 is the measure of the difference between a taste pair, which we call auROC_D. A 250 ms sliding window with 50 ms steps was applied to taste-evoked activity, and the auROC_D was calculated for every pair, the PI was defined as, $PI = < D >_{\text{opposite}} - < D >_{\text{same}}$, where $< D >_{\text{opposite}} = \frac{1}{4}(auROC\_D_{\text{SQ}} + auROC\_D_{\text{SC}} + auROC\_D_{\text{NQ}} + auROC\_D_{\text{NC}})$, and $< D >_{\text{same}} = \frac{1}{2}(auROC\_D_{\text{SN}} + auROC\_D_{\text{CQ}})$; subscripts mean taste pairs (e.g., SN means the taste pair of S and N). The PI ranges from -0.5 to +0.5, with positive value indicating palatability coding and negative indicating anti-palatability coding. Significance was based on a 95% confidence interval of baseline PI distribution, which was assessed by pooling PIs of all units before passive taste deliveries (from -1 to 0 s according to taste delivery).

6.2.7. Time Course of Taste Identity Coding

To quantify the time course of taste coding, we analyzed data relying on a sliding window procedure. A 250 ms window and 50 ms steps were used. Two distinct but convergent analyses were used. First, a one-way ANOVA test was used to compare activity across tastants to determine if a neuron was taste-specific in a specific bin ($p < 0.05$, with Bonferroni correction). The first significant bin after taste delivery was defined as the onset of taste coding. The percentage of units that showed significant modulations within each bin was computed. Differences between groups were assessed on the basis of two proportion test ($p < 0.05$). For bins

with number smaller than 5, Fisher exact test is used ($p < 0.05$). The second method used to analyze the time course of taste coding relied on the taste classification procedure (see 6.2.4) applied on each time bin. The decoding performance described how well a given taste was encoded by a neuron at a given time window.

6.2.8. State Dependent Modulation of Taste Responses

To determine if a neuron was modulated by the anticipatory state of the animal, we compared the taste evoked activity between Self and Passive conditions. To determine the effects of the anticipatory state on general firing activity, firing rates averaged across the entire 2.5 s analysis period were compared. A neuron was defined as significantly modulated when at least one of the tastants showed a significant difference across conditions (paired-sample t-test, $p < 0.05$). To determine if the anticipatory state modulated neurons' responsiveness to the four tastants, we used a 3-way ANOVA test (taste [4] X time [10] X condition [2], 250 ms bin size was used). A significant state modulation was defined by $p < 0.01$ for at least one of terms containing condition (i.e.: condition, time X condition, taste X condition, taste X time X condition). The time course of the modulation was assessed by modulation index (see 6.2.9).

6.2.9. Modulation Index (MI)

To assess the difference between responses to Self and Passive over time, we relied on a modulation index (MI). First, we calculated the auROC value (see 6.2.1) between two responses to the same tastants delivered in the two conditions, using a 250 ms moving window (50 ms step). Then, we defined the MI by subtracting 0.5 from the auROC value. As a result, MI ranged from -0.5 to 0.5, with 0 meaning no difference. Positive MI indicated an increase of firing activity of Self versus Passive, whereas negative indicated a reduction. For each response, significant modulations were determined on the basis of a 95% bootstrapped CI, obtained by shuffling trials between the two conditions. The absolute value of MI was used as a measure of absolute change. In the case of population MI, significance for taste responses over time was determined by comparing the population MIs at a given bin to baseline MIs (independent-samples t-test, $p < 0.05$, $N = 328$). The baseline MIs were calculated on the basis of spiking activity before passive deliveries and cue onsets.

6.2.10. Principal Component Analysis (PCA) on Response Patterns

Principal component analysis (PCA) was used to analyze the pattern of stimulus-evoked response dynamics. The use of PCA is based on (Narayanan & Laubach 2009). Temporal patterns were analyzed for all the significant taste responses. A response X time (250 ms bin, from -1 to 2.5 s for taste-evoked responses; 50 ms bin, from -0.5 to 0.5 s for cue-evoked responses) matrix of auROC normalized responses was compiled. A PCA was applied to the matrix. Principal components accounting for more than 5% of the variance were selected. The eigenvectors of selected PCs were used to describe the temporal dynamics of taste responses. In Figure 2.2D, taste responses were sorted using values of PC1, 2 and 3, according to a hierarchical cluster tree based on pairwise Euclidean distance.

6.2.11. Analysis of Local Field Potential (LFP) Data

LFP data analyses relied on the Chronux toolbox (http://chronux.org/). LFP signals across recording channels within the same session were pooled together. LFP traces containing artifacts were discarded. The power spectral density was calculated during 1 s before cue onsets for the trials of Respond and Ignore conditions for each session. Alpha band was determined as 8-14 Hz (Bastos et al. 2014; Haegens et al. 2012; Haegens, Händel, et al. 2011; Haegens, Nácher, et al. 2011; Jensen et al. 2014). Significance was determined by Wilcoxon rank test with Bonferroni correction ($p < 0.05$).

6.2.12. Analysis of Electromyography (EMG) Data and Mouth Movements

EMG data were first rectified. A detection algorithm based on statistics from baseline period was used to identify the onset of mouth movement. The baseline period was defined as 0.5 s before cue onsets or passive taste deliveries respectively. To exclude occasionally spontaneous mouth movements during baseline, trials containing any data point exceed the 40% of the maximum EMG signal in the session was removed. The detection threshold was defined as the mean plus 3 times standard deviation of all data points in the baseline period. The time point where the average of EMG signals of a session exceeded the threshold was defined as the mouth movement onset.

To validate the detected onsets by the detection algorithm, a second method based on visual inspection of EMG traces was used. Suggested by visual observation and previous studies (Travers & Norgren 1986; Travers et al. 1987), conditional mouth movements are represented by a ramping of EMG signals and happens right before receiving self-administered or conditionally delivered taste solutions (Samuelsen et al. 2013; Samuelsen et al. 2012; Gardner & Fontanini 2014). In EMG traces, it is distinguishable from others mouth movements. Thus, we visually identified the beginning of conditional mouth movement as the onset for each trial. Trials failed to show this signature were excluded.

6.2.13. Event-related Modulation in the Pavlovian Conditioning Task

For analysis of event-related modulation, each trial was divided into “pre-cue” (before cue onsets), “cue” (first 10 s of cue), “food” (last second during cue with food pellet dropped), and “consumption” (5 s window after cue offsets). Firing rates during cue, food, and consumption periods were compared with the same length of baseline activity in the pre-cue period (e.g., −10 to 0 s, according to cue onsets, was used as the baseline for each cue period). A paired-samples t-test was used to determine responsiveness ($p < 0.05$). To calculate population peristimulus time histogram (PSTH) and modulation magnitude, a normalization procedure according to the area under the receiver operating characteristic curve (auROC) was used (see 6.2.1). The modulation index (MI) was defined on the basis of the auROC and was computed by simply subtracting the baseline value of 0.5 from the auROC value. This procedure was done to represent stimulus-evoked activity on a more intuitive −0.5 to +0.5 range, with positive values meaning an increase of activity and negative values meaning a decrease of activity. For population PSTHs, a 1 s bin was used and baseline firing was computed on the basis of 10 bins before the cue onset. Cue response peaks or troughs were determined by using 1 s bins. Head entry-related modulation during the cue and the waiting time were calculated. Because the inter-poke interval appeared to be longer than 250 ms, we used 250 ms bins to compare firing rates

before and after head entries. An unbalanced two-way ANOVA (before or after head entries either during the cue or waiting time) was applied. Groups that are modulated by head entry or context (i.e., during cue or waiting time) and the interaction of the two were defined ($p < 0.05$). A *post hoc* multiple comparison with Bonferroni correction was used to compare modulations due to head entries during the cue with those observed during the waiting time ($p < 0.05$ with correction). The Fisher exact test was used to compare the proportions of different groups. MIs of head entries were calculated by comparing 250 ms activity before and after head entries for each context. Absolute MIs of the two groups were compared to determine if they were different (independent-samples t-test, $p < 0.05$).

6.2.14. Trail-by-trial Variability

The trial-by-trial variability was assessed by the Fano factor, which is the ratio between the variance and mean (Fano factor = variance/mean) (Churchland et al. 2010). We calculated the Fano factors using 250 ms neural activity before and after head entries in the Pavlovian conditioning task. The Fano factors were then compared using independent-samples t-test.

6.2.15. Cue Response and Head Entry Rate Onsets in the Pavlovian Conditioning Task

We applied a moving window method to estimate cue response onsets. Neural activity from −10 to 10 s around cue onsets was computed with a 1 s bin, which moved by 100 ms steps. The 99% confidence interval (CI) based on t distribution was drawn from the baseline period (−10 to 0 s), and the first bin after 0 that exceeded the upper CI or fell below the lower CI was defined as the cue response onset of the excitatory response or inhibitory response, respectively. The same procedure was used for determining head entry rate onset.

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