Published in final edited form as:

Prog Neurobiol. 2008 November ; 86(3): 245–263. doi:10.1016/j.pneurobio.2008.09.003.

Cracking Taste Codes by Tapping into Sensory Neuron Impulse Traffic

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Abstract

Insights into the biological basis for mammalian taste quality coding began with electrophysiological recordings from “taste” nerves and this technique continues to produce essential information today. Chorda tympani (geniculate ganglion) neurons, which are particularly involved in taste quality discrimination, are specialists or generalists. Specialists respond to stimuli characterized by a single taste quality as defined by behavioral cross-generalization in conditioned taste tests. Generalists respond to electrolytes that elicit multiple aversive qualities. Na⁺-salt (N) specialists in rodents and sweet-stimulus (S) specialists in multiple orders of mammals are well-characterized. Specialists are associated with species’ nutritional needs and their activation is known to be malleable by internal physiological conditions and contaminated external caloric sources. S specialists, associated with the heterodimeric G-protein coupled receptor: T1R, and N specialists, associated with the epithelial sodium channel: ENaC, are consistent with labeled line coding from taste bud to afferent neuron. Yet, S-specialist neurons and behavior are less specific than T1R2-3 in encompassing glutamate and E generalist neurons are much less specific than a candidate, PDK TRP channel, sour receptor in encompassing salts and bitter stimuli. Specialist *labeled lines* for nutrients and generalist *patterns* for aversive electrolytes may be transmitting taste information to the brain side by side. However, specific roles of generalists in taste quality coding may be resolved by selecting stimuli and stimulus levels found in natural situations. T2Rs, participating in reflexes via the glossopharyngeal nerve, became highly diversified in mammalian phylogenesis as they evolved to deal with dangerous substances within specific environmental niches. Establishing the information afferent neurons traffic to the brain about natural taste stimuli imbedded in dynamic complex mixtures will ultimately “crack taste codes.”

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Keywords

Taste quality; gustatory coding; chorda tympani nerve; glossopharyngeal nerve; geniculate ganglion; specialist neuron; generalist neuron; response patterns; labeled lines; species differences; sodium appetite; mixture suppression; salty taste; sweet taste; ionic tastes; non-ionic bitter tastes; taste mixtures; adaptation

1. Introduction

Taste identifies nutritious and poisonous substances. This review focuses primarily on what is known about the way in which mammalian peripheral taste neurons encode information about taste quality (Nowlis and Frank, 1981); we also comment on preference (valence); both qualities and preferences vary in intensity. Encoding begins with molecular taste receptors (TR) in plasma membranes of taste-bud receptor cells (TRC), which activate afferent neurons that signal qualitative differences among a large and chemically diverse set of compounds. Many can be condensed into a limited number of perceptually similar categories once oral sensations derived from olfactory and trigeminal receptors, which contribute to overall sensory experience and influence decisions to ingest or reject stimuli, are excluded. Sugars, salts, acids and bitter substances form the four taste categories most frequently used as standard stimuli in scientific investigations of taste in humans and other mammalian species. It is likely no coincidence that corresponding perceptual categories of sweet, salty, sour, and bitter are intrinsically related to naturally consumed major nutrient groups (e.g., carbohydrates, salts) and harmful substances (e.g., toxins, acids) avoided by mammals and other vertebrates.

Elemental components of other important nutrients, protein and fat, are free amino acids and fatty acids, respectively, which enhance palatability of food and stimulate consumption. For example, glutamate, a free amino acid found in many foods, meat and fish included, may be added to enhance the flavor of a cuisine. The unusual taste quality elicited by L-glutamate is described as “savory,” commonly called “*umami*” from the Japanese (Yamamoto *et al.*, 1988; 1991; Hettinger *et al.*, 1996; Galindo-Cuspinera and Breslin, 2006; McCabe and Rolls, 2007). Also, lingual lipase is present in the oral cavity to rapidly break down fats into free fatty acids, possibly the critical agents for eliciting a fat “taste” (McCormack *et al.*, 2006). *Umami* taste is increasingly included on the list of taste categories, as is fat, although to a far lesser extent. Despite their ability to enhance palatability, the inclusion of *umami* and fat as taste categories remains controversial. Yet there has long been general agreement on sweet, salty, sour and bitter as unique human taste experiences (McBurney and Gent, 1979) and this perspective has strongly influenced research in other species.

Taste activation by nutritious and poisonous substances has a powerful influence on physiology and behavior. Taste stimuli trigger changes in autonomic and endocrine activity, and elicit somato-motor reflexes that foster or terminate consumption. For example, besides taste sensations, taste stimuli elicit salivation (Bradley *et al.*, 2005) and cephalic insulin release (Teff, 2000) to assist digestion, or oral-motor ejection reflexes to prevent swallowing of toxins, a primary province of CN IX afferents in rats (King *et al.*, 2000). And, stimulation of pharyngeal and laryngeal taste buds activate CN X afferent limbs of protective reflexes (Bradley, 2000). Factors like physiological state and stimulus context have substantial impact on the strength of signals transmitted by peripheral taste nerves (Contreras and Frank, 1979; Formaker *et al.*, 1997). Apparently, in real life, sodium salt and sugar tasting are regulated by need for Na⁺ and an uncontaminated carbohydrate source.

The rodents: rat, hamster and mouse, the foremost research models for taste and its effects on ingestion, and new and old world primates, including humans, have provided critical

knowledge about taste coding mechanisms. Much of this knowledge comes from parallel studies using electrophysiological recording of whole nerve and single-neuron responses and psychophysical studies of taste-mediated behavior. We will draw heavily upon the literature using this two-barreled approach to address peripheral coding mechanisms of taste. In mammals, the chorda tympani (CT) branch of cranial nerve (CN) VII innervates taste buds in fungiform papillae distributed across the anterior tongue, the greater superficial petrosal (GSP) branch of CN VII innervates palatal taste buds, and the lingual branch of the glossopharyngeal (GL), CN IX, innervates taste buds in foliate papillae of the posterior-lateral and circumvallate papillae of the posterior-medial tongue. Rat CN IX and CN VII overlap in the anterior clefts of the foliate papillae (Miller *et al.*, 1978). Much of the experimental literature on peripheral taste coding arises from work involving the CT nerve and its geniculate ganglion. This review, therefore, reflects that emphasis. Humans appreciate all taste qualities when stimuli are applied to either anterior or posterior tongue regions (Grover and Frank, 2008); however rats without taste information transmitted by CN VII fail to discriminate taste-stimulus quality (Spector & Grill, 1990). Information transmitted by the rat CT also supports mouth “gapes” attributed to learned sucrose aversions (Eylam *et al.*, 2000); and, after rewiring the CT to GL receptive fields (King *et al.*, 2008), information supporting aversive reflexes primarily transmitted by the normally wired lingual branch of CN IX (King *et al.*, 2000). A similar conversion shown for sensory function with rewiring in rats and mice was interpreted as a possible selective coupling between afferent and peripheral target (Oakley, 1967, Ninomiya, 1998) that would be useful for maintaining consistent information transmission during normal TRC turnover.

Although taste qualities distinguished by people (McBurney and Gent, 1979) strongly influenced interpretations of classical taste nerve recordings, the first recordings also anticipated the distinctive taste worlds of different species (Anderson *et al.*, 1950; Beidler *et al.*, 1955; Pfaffmann, 1955), nations reflecting their individual genomes (Shi *et al.* 2003; Shi and Zhang, 2006). However, they did not anticipate discovery of distinct sets of species-dependent TR that had stimulus chemistries consistent with discrete sweet, glutamate, bitter and sour taste qualities (Chandrashekar *et al.*, 2006). Interpretation of the 21st century findings in the context of characteristics of CT neuron types, discovered electrophysiologically, concludes this review.

2. Classic Recordings and the Coding Controversy

When it first became possible to “tap in on the electrical impulse traffic in the sensory nerves” (Pfaffmann, 1984), the function of an individual mammalian taste neuron was a puzzle. Electrophysiological recordings from taste nerves of cats, dogs, domestic rabbits, lab rats and golden hamsters did not reveal 4 functional classes of single-nerve fibers, one for each of the 4 taste qualities expected from human taste sensation; i.e. 4 “labeled lines.” Single- and few-fiber preparations of peripheral axons teased apart from desheathed taste nerves (Fig. 1A) were clearly not functionally identical in stimulus chemistry but were divisible into two populations: narrowly tuned neural specialists and broadly tuned neural generalists (Pfaffmann, 1941; Zotterman, 1935). Specialists responded specifically to prototypic stimuli of one quality, such as sweet sucrose or salty NaCl (Anderson *et al.*, 1950; Pfaffmann, 1955; Fishman, 1957) but generalists responded to stimuli that had distinctly different tastes to humans, for example, salty sodium chloride and sour hydrochloric acid (Pfaffmann, 1941; 1955; Fishman, 1957). Furthermore, nerve fibers sampled from nerves of multiple mammalian species differed a great deal. There were no sucrose specialist neurons in cat as in dog (Cohen *et al.*, 1955; Anderson *et al.*, 1950) and there were no NaCl specialist neurons in rabbit, as in murid and cricetid rodents (Pfaffmann, 1955; Fishman, 1957). The great variability in stimulus chemistry across single neurons of a given species and, more so, across various species, was bewildering and led to introduction of a “whole-nerve” recording technique and an “across-fiber” theory of taste coding.

In 1953, Beidler introduced electronic integration into taste-nerve recording, providing an efficient and quantitative means for measuring the summed activity from all fibers in a taste nerve at once (Beidler, 1953). This valuable technique was used to assess species differences (Beidler *et al.*, 1955) and obtain stimulus concentration-response functions, data used to draw inferences about basic physical-chemical principles operating between taste stimuli and receptors (Beidler, 1954). Importantly, the whole-nerve technique also allowed study of neural adaptation across time with continuous stimulus application, a profile of neural spiking with initial transient and subsequent steady state epochs (Pfaffmann and Powers, 1964). It was discovered that taste adaptation to NaCl in rats is clearly partial, with a CT high-level steady state maintained indefinitely (Fig. 1B); in contrast, neural and behavioral adaptation in humans, is complete in less than one minute (McBurney and Pfaffmann, 1963; Diamant *et al.*, 1965). Whole-nerve recording remains an invaluable technique for assessing taste function today. Both for its original purposes and, lately, in tandem with behavioral tests of taste preferences, whole-nerve recordings provide excellent functional tests of taste loss/gain resulting from the manipulation of TR by genetic engineering (Chandrashekar *et al.*, 2006). However, in spite of the convenience, it obscures in one measure the neuronal activities of functionally distinct sets of chemosensory neurons.

The broad tuning of generalist populations of single neurons across stimuli representing the 4 human basic tastes inspired the resilient pattern theory of taste quality coding (Pfaffmann, 1959). The theory stipulated that it is “the relative amount of ...parallel activity across nerve fibers...that is important.” Continuous taste-stimulus dimensions and a continuum of taste qualities, rather than discrete taste qualities, were proposed based on overlapping individual rat CT neuron response profiles (Erickson *et al.*, 1965). This work presaged the current routine use of multivariate statistics (Bieber and Smith, 1986) to help interpret complex single-neuron data (e.g., Breza *et al.*, 2006; Yoshida *et al.*, 2006). Pattern theory is an historical antecedent of what is now called distributed coding, which contrasts with the labeled-line coding promoted by results of genetic manipulations on peripheral neural activity and behavior toward taste stimuli (Chandrashekar *et al.*, 2006). Yet, a question remains: “Are taste qualities coded by dedicated neurons or patterns of neural activity distributed across all the neurons?” Perhaps the answer lies in where we tap into taste pathways.

3. Best-Stimulus–Behavior Consonance

The golden hamster (*Mesocricetus auratus*) is a valuable alternate to the ubiquitous laboratory rat as a rodent model for taste studies because the hamster CT responds more equally to sucrose, quinine hydrochloride, NaCl and HCl. The rat CT responds well only to the ionic stimulus prototypes: those representing the human salty and sour qualities and less well to sweet and bitter stimuli (Beidler *et al.*, 1955; Lundy and Contreras, 1999; Breza *et al.*, 2006; 2007). Outbred lab golden hamsters, originating from one captured litter, would also be expected to show less individual genetic variation (Frank *et al.*, 2004) than lab rats. The CT was the nerve of choice because, in contrast to the GL, the CT has a convenient anterior tongue receptive field and the majority of CT neurons respond to taste stimuli (Lundy and Norgren, 2004). The GL is mostly general sensory.

However, detailed coding models were developed based on rat CT data that primarily addressed coding of ionic taste stimuli (Erickson *et al.*, 1965), still an unresolved topic. A change in rodent species, and using midrange, rather than high, concentrations of multiple stimuli of each quality (Frank, 1973), had surprisingly enduring effects on conceptualizing taste quality coding. Contributing to that endurance was the later appreciation of measures of generalization of conditioned taste aversions (CTA) (Palmerino *et al.*, 1980) to assess stimulus similarities in animals (Nowlis, 1974; cf., section 5). CTA generalization does not directly address whether stimuli can be discriminated from one another, which is more difficult to establish but

unnecessary for identification of quality. Furthermore, multivariate analyses: multidimensional scaling, factor analysis and cluster analysis, already used to help interpret people's judgments (Schiffman and Erickson, 1971), were introduced as aids for interpreting complex gustatory behavioral (Nowlis and Frank, 1981) and neural (Frank *et al.*, 1988) datasets.

Single hamster CT neurons respond to sucrose, NaCl, HCl and quinine HCl, 4 prototypic taste stimuli, with 3 characteristic activity profiles (Frank, 1973). Afferent neurons responding best to sucrose or best to NaCl responded little or inconsistently to the other 3 stimuli. However, CT neurons responding best to HCl also consistently responded to NaCl and quinine HCl. No CT neurons responded best to moderate concentrations of quinine. Hamster CT-neuron, best-stimulus, evoked response rates ranged from 4 to 60 spikes per sec, more than an order of magnitude. The variation may be related to stage of reconnection between afferent fibers and TRC or TRC maturity, given the 10-day TRC turnover period (Shimatani *et al.*, 2003). Neurons with low response rates, and likely lower impact downstream, were least specifically tuned across stimulus quality (Frank, 1973).

How individual peripheral neurons transmit consistent information to the brain with this changeability, nevertheless, remains a mystery. Albeit, at any point in time, a handful of dedicated mouse CT neurons exclusively innervate single fungiform papillae, each containing one taste bud, forming "bud units"; a precision maintained in mice over-expressing BDNF with twice the neurons and one third the taste buds (Zaidi and Whitehead, 2006; Zaidi *et al.*, 2007). Many hamster CT neurons branch to innervate multiple papillae, although a single taste bud is also found in each of their fungiform papillae (Whitehead *et al.*, 1985) and the GG peripheral CT-fiber to taste bud ratios are about equal for the two rodents. The value of the different patterns in the 2 rodent species is unknown. There are many other strategies, given fungiform papillary designs with multiple buds per papilla, and bud number varying with papilla size and location in species like sheep, pig and rhesus monkey (Mistretta *et al.*, 1988; Bradley *et al.*, 1985; Mack *et al.*, 1997; Kumar and Bate, 2004). Diversity in lingual taste bud arrangement likely evolved to function in mouths of animals with grossly different modes of eating and drinking.

Characteristic response profiles in hamsters and other species came to be known as sucrose-best and NaCl-best specialists because of response specificity, and HCl-best generalists because they responded to stimuli with qualities that are distinctly different to people. The neurons with these response profiles are known as S (*sucrose*), N (*NaCl*) and E (*electrolyte*) types (Frank *et al.*, 1988; Ninomiya and Funakoshi, 1988). Traits of rodent CT S, N, and E type neurons are listed in Table 1.

3. 1. Sweeteners, S-type Neurons

The S neurons (Fig. 2) are about one quarter of hamster CT neurons (Table 1, col. 1) and respond primarily to sweeteners (col. 2). CT neurons with similar sucrose-best response profiles have been described for other species (cf., section 4). They burst with a 1.4 Hz "sweet rhythm" to sweeteners as well as mixtures containing sweeteners (Frank *et al.*, 2005). CT S neurons are rare in the lab rat CT (Frank *et al.*, 1983); but despite their 6–11% frequency, rat S specialist CT neurons, recorded by microelectrode from the geniculate ganglion (GG), respond robustly and selectively (Lundy and Contreras, 1999; Breza *et al.*, 2006; 2007). With the GG preparation, single-neuron responses are recorded from either GSP or CT neurons depending on where stimuli are applied, without severing afferent nerve fibers from neuronal soma. Sucrose specialists are more common when rat palatal taste buds are stimulated (Nejad, 1986; Harada *et al.*, 1997; Sollars and Hill, 2005).

A core of sugars, some amino acids, some artificial sweeteners (compounds honed by food chemists to the human sweet taste) like saccharin but not aspartame, activate hamster CT S

neurons (Frank *et al.*, 1988). These chemically diverse compounds all elicit sweet taste in humans, and are behaviorally cross-generalized specifically to sucrose by hamsters (Fig. 3) and mice (Nowlis *et al.*, 1980; Ninomiya *et al.*, 1984; Frank and Nowlis, 1989) in CTA tests. CT neurons that respond selectively to sucrose (Fig. 2) also respond vigorously to stimuli of varied molecular structure that taste like sucrose to rodents, results that together indicate that the organization of rodent peripheral gustatory systems is quality specific, at least for sweet taste.

3. 2. Ionic Taste Stimuli

The results for ionic taste stimuli are more complex, as are their tastes to humans (Frank *et al.*, 2001). About three-quarters of hamster and nine-tenths of rat CT neurons (Lundy and Contreras, 1999; Breza *et al.*, 2006; 2007) respond well to ionic taste stimuli (Fig. 4); most respond to NaCl, the prototypic salt stimulus used in nearly all electrophysiological taste investigations. HCl, a mineral acid commonly used as the sour prototype, is also often effective. About one-half of hamster CT neurons respond primarily to sodium salts (Table 1, col. 1), displaying a specialist's NaCl-best activity profile (Fig. 4, blue bars). About one-quarter are electrolyte generalists (Table 1, col. 1) showing the HCl-best response profile (Fig. 4, red bars); although most responsive to 3 mM HCl (Frank, 1973), they also respond to many inorganic salts, including NaCl, MgSO₄, KCl and especially NH₄Cl, as well as organic acids and the ionic bitter prototype: quinine HCl.

3. 2.1. N-type Neurons—CT N-type neurons have been described for multiple rodents: rats, hamsters, mice and gerbils (Frank *et al.*, 1983; 1988; Ninomiya *et al.*, 1984; Somenarain *et al.*, 1992), as well as sheep in which they have characteristic small receptive fields (Nagai *et al.*, 1988). A subset responds substantially to HCl. N-neuron HCl responses increase after repeated application of multiple stimuli (Frank *et al.*, 1983; Frank *et al.*, 1988); the activation may be an experimental artifact reflecting a sensitization due to disruption of lingual ion transport mechanisms (Boudreau *et al.* 1983). TRC acidification and changes in cell volume are correlated with CT responses to acids (Lyll *et al.*, 2001; 2006). N neurons are cation specific (Table 1, col. 3) and responses to electrolytes such as MgSO₄ or KCl and NH₄Cl are rarely above baseline (Fig. 4). Na⁺-specialist GG CT neurons also respond weakly, if at all, to non-sodium salts (Lundy and Contreras, 1999; Breza *et al.*, 2006; 2007). Accordingly, equimolar NaCl/ KCl response ratios readily distinguish mouse and rat N from the E neurons (Yasumatsu *et al.*, 2003), as do NaCl/NH₄Cl response ratios in sheep (Nagai *et al.*, 1988). Another distinguishing characteristic of rat GG Na⁺-specialists is their increase in response rate with cooling from 25° to 10°C after minutes of adaptation and reciprocal decrease with warming from 10° to 25°C (Lundy and Contreras, 1999; Breza *et al.*, 2006; Table 1, col. 7).

Rodent CT neurons with NaCl-best profiles respond selectively to Na⁺/Li⁺ salts compared to other prototypic taste stimuli and non-Na⁺/Li⁺ salts. Response selectivity towards NaCl is also evident behaviorally using CTA tests of taste similarity. As shown in Fig. 5, Na⁺ salts cross-generalize with each other, but not with non-Na⁺ salts, sour or bitter tasting compounds. The pattern of results for salty stimuli parallels the specificity for sweet-tasting stimuli noted above and indicates rodent Na⁺/Li⁺-specialists form another discrete physiological group that responds selectively to one behaviorally verified stimulus class.

3. 2.2. E-type Neurons—Rodent CT electrolyte-generalist stimulus chemistry is discordant with a single taste quality. Besides NaCl and other inorganic salts, E neurons respond to HCl and ionic bitter stimuli like quinine HCl, hedonically negative stimuli with distinguishable chemosensory qualities (Fig. 4). CT E neurons, like all GL electrolyte-sensing neurons (Kitada *et al.*, 1998), are salt cation generalists but respond selectively to anions (Formaker and Hill, 1988; Rehnberg *et al.*, 1993; Frank, 2000) (Table 1, col. 4). Response rates decrease with

increasing anion size more dramatically in E than N neurons. Although E neurons show a preference for H^+ , they are a diverse group that is not specific for acidic compounds. They are the only CT neurons to significantly respond to the bitter prototype, 1 mM quinine HCl (Table 1, col. 6), which hamsters find aversive (Frank *et al.*, 2004). Yet, CT E neuron responses to 1 mM quinine in hamsters (Frank, 1973; Frank *et al.*, 1988) and 20 mM quinine in rats (Frank, 1983) are relatively weak. As a group, rodent CT electrolyte generalists seem to be non-specific detectors of aversive electrolytes.

Gustatory systems may not precisely discriminate among aversive electrolytes; in fact, hamster behavioral generalizations among acids and bitter salts overlap considerably (Fig. 5). Peripheral taste neurons, like rat brainstem taste neurons (Chen and Di Lorenzo, 2008), may naturally respond to multi-quality aversive stimuli. Rodents are assisted in discriminating among aversive chemosensory stimuli by sensations derived from trigeminal sensory nerves (Wang *et al.*, 1993; Pittman and Contreras, 1998); and, reflex salivation, elicited by sour tasting acids (Matsuo and Yamamoto, 1989; Bradley *et al.*, 2005), may contribute additional taste and texture cues to amplify or modify ongoing acid-elicited CT responses. Besides, cues for bitter likely include activation of GL quinine-best specialists (Frank, 1991) (Fig. 1A) and, subsequently, the GL-activated “bitter-best” neurons in the brainstem nucleus of the solitary tract (NST; Geran and Travers, 2006), circuits that may participate in oral motor-reflexes (King *et al.*, 2000; Travers and Travers, 2005; cf., section 4 and section 5).

Because the great majority of electrolyte-sensing afferent neurons respond well to NaCl, CT N and E-types alike, relative effects of other, more discriminating, compounds, like KCl (Yasumatsu *et al.*, 2003, see above), or originally HCl (Frank, 1973), were used to distinguish between types. This primacy of NaCl as a rodent taste stimulus, a puzzle for quality coding, is a reason to test mid-range electrolyte concentrations. For example, in rat E-neurons, 5-sec responses to 100 mM NaCl are twice the rate of responses to 1 mM HCl. Also, the time epoch over which a response is measured can determine whether HCl or NaCl is most effective. E-neuron, early, short (1–2 sec) peak responses to 100 mM NaCl and 10 mM HCl are equal; whereas, lengthier later steady-state responses show NaCl is one-fifth as effective. Hamster CT E-neurons respond transiently to NaCl and steadily to HCl (Frank *et al.*, 1983; Hettinger and Frank, 1990), and in rat and mouse, the E-neuron 1 mM NaCl threshold is lower than the N neuron 10 mM NaCl threshold (Frank *et al.*, 1983; Yasumatsu *et al.*, 2003) (Table 1, col. 5), which more closely approaches rat salivary Na^+ levels (Contreras & Catalanotto, 1980).

Together with the GG S-specialists and Na^+ -specialists described above, the rat GG recordings confirm the 3 classes of CT response profiles discovered with hamster CT nerve-fiber recording (Frank, 1973). Rat electrolyte generalist GG CT neurons, which respond well to NaCl, non- Na^+ halide salts, acids and quinine HCl, include distinct clusters with different activity profiles (Lundy and Contreras, 1999; Breza *et al.*, 2006; 2007). One cluster of GG CT electrolyte generalists, ~37 %, responded clearly to quinine and NaCl. Another cluster, ~63 %, responded well to citric acid and NaCl, which was activated by cooling (40° to 10°C) as were Na^+ -specialists (Breza *et al.*, 2007; Table 1, col. 7). CT E neurons may be a conglomerate of several sub-categories, all of which, interestingly, respond to NaCl, applied to a water-rinsed tongue. The thermal specificities bring to mind humans tasting cooling as sour-salty (Cruz and Green, 2000), which suggests CT-neuron responses elicited by thermal stimuli (Ogawa *et al.*, 1968) may be interpreted as tastes by the brain, as may CT-neuron responses elicited by mechanical stimuli (Matsuo *et al.*, 1995; Lim and Green, 2008). However, behavioral confirmation for distinct taste, or multi-modal, coding roles of the generalist clusters, the majority of rat GG neurons, is needed.

Future research may identify additional specific neuronal types with a more diverse, species-specific and judicious choice of stimuli or discovery of substances, such as amiloride, that

selectively modify unique receptor mechanisms (Contreras & Lundy, 2000). Amiloride blocks the Na⁺-specific epithelial Na⁺ channel ENaC and the Na⁺-specific taste in rodents (Fig. 1B, DeSimone and Ferrell, 1985; Brand *et al.*, 1985; Hettinger and Frank, 1990; Ninomiya and Funakoshi, 1988; Halpern, 1998; Schiffman *et al.*, 1990; c.f., section 4 and section 5). HCl, a strong mineral acid not likely to be encountered during natural foraging is injurious to the plasma membrane, which may contribute to response “non-stationarity” (Boudreau *et al.*, 1983), i.e., changes in mean, variance and autocorrelation structure over time, during experimental recording. The HCl-responsive, 70%, are a larger proportion of GG generalist neurons than are the citric-acid responsive, 32% (Lundy and Contreras, 1999; Breza, *et al.*, 2007); and use of citric acid as the sour stimulus instead of HCl revealed the small populations of vigorously reactive rat CT GG neuron types differentially responsive to 10 mM citric acid and 20 mM quinine (Breza *et al.*, 2007) noted above. Finer classification of physiological quality-specificity in afferent neuron populations may establish a better understanding of electrolyte taste coding as it has for itch coding (Johanek *et al.*, 2007; Namer *et al.*, 2008).

3. 3. Summary

Midrange concentrations of 4 prototypic stimuli representing human taste qualities and 5-sec response measures revealed 3 types of hamster CT afferents categorized by best stimulus. Albeit great E-neuron diversity, subsequent, multivariate statistical analysis showed that S, N and E neurons’ responses to taste stimuli have distinct stimulus chemistries. Multivariate analysis of behavioral generalization profiles identified a sucrose-like detection, homologous to human sweet, and 3 other discriminations that hamsters make when tasting a battery of ionic stimuli that activate N and/or E neurons, possibly homologous to general distinctions that humans make among the qualities of acids, bitter salts and sodium salts; i.e., sour, bitter and salty tastes.

Specialist S and N afferent neurons allow for the specific behavioral detection of sweeteners and sodium salts, respectively. However, there is far more uncertainty about peripheral coding mechanisms underlying sour and bitter taste discrimination. Part of this uncertainty lies in failure to identify CT neuron types specialized for sour and bitter stimuli, which activate generalist neurons that also respond to NaCl. Hamsters behaviorally cross-generalize ionic stimuli that affect E neurons with HCl and/or quinine HCl but not NaCl, making CT neurons more broadly-tuned than the animals’ behavior predicts.

4. Species and Inbred Strains Differ in What They Can Taste

It is increasingly clear that there are profound species differences in taste stimulus chemistry. Knowledge of a species ecological niche, that is, what the species has available to sample and decide whether or not to eat or drink, is needed to understand what a species must code as tastes. A model developed by Boudreau more than 20 years ago has facial nerve (CN VII) systems composed of four distinct stimulus detectors (modules, qualities); however, a given species, be it rat, cat, goat or dog, may not have all four detectors; and, for each species, detection is distinct and consonant with its ecological niche (Boudreau *et al.*, 1985). Also, studies of variation in inbred strains of rodents, especially laboratory mice, seek independently assorting genes coding for the set of distinct taste detecting systems (Lush, 1991). Inbred strains demonstrate the availability of a wide within-species variation in taste behaviors.

4. 1. Sweet Tastes

CT S neurons have been characterized in multiple mammalian species, including various primates (Sato *et al.*, 1975; Hellekant *et al.*, 1997a; 1997b; 1998; Danilova *et al.*, 2002; Danilova and Hellekant, 2004) and also in multiple strains of inbred mice (Ninomiya *et al.* 1984b). Chimpanzee CT neurons that respond best to sucrose also respond to all stimuli that

are sweet to humans (Hellekant *et al.*, 1997b). This precise congruence between chimpanzee CT neural responses and human taste sensation is seen for no other species: hamster, pig (Danilova *et al.*, 1998; 1999), new-world or other old-world primate (Hellekant *et al.*, 1998). Regardless of species, however, S-neuron activation accurately predicts which compounds taste sucrose-like (Danilova and Hellekant, 2004; Table 1, col. 2). An example of correspondence between neural responsiveness and behavior involves the amino acid, D-phenylalanine (D-Phe). Hamster CT S neurons are highly responsive to D-phe (Hyman and Frank, 1980) and hamsters behave as if this stimulus tastes sweet, like sucrose (Nowlis *et al.*, 1980). Ninomiya and his colleagues (1984a; 1984b) studied the neural responses and behavior of inbred strains of mice. In strains in which D-Phe tasted similar to sucrose, CT S neurons responded to D-Phe, but in strains in which D-Phe tasted different from sucrose, CT S neurons were unresponsive to D-Phe (Ninomiya *et al.*, 1984b). Allelic variation (Inoue *et al.*, 2004) in *Tas1r3*, a gene located within the mouse *Sac* locus on chromosome 4 (Bachmanov *et al.*, 2001) may underlie this strain-specific distinction in CT S neuron and behavioral responses to D-Phe (Ninomiya *et al.*, 1987; Capeless and Whitney, 1995). *Sac* codes for the T1R3 moiety of the heterodimeric G-protein coupled (GPCR), candidate sweet receptor: T1R2–T1R3 (Zhao *et al.*, 2003).

In humans, orthologous sweet receptor genes are located on syntenic chromosome 1 (Liao and Schultz, 2003), and the coded heterodimeric receptors, which interact at binding sites on multiple receptor domains (Assadi-Porter *et al.*, 2008) with all natural and synthetic compounds that are sweet to humans, are expressed in fungiform papillae. Orthologous sweet receptor genes are also found on cat chromosome C1, and T1R3 is expressed in taste papillae; however, *Tas1r2* is a pseudogene (Li *et al.* 2006). Consequently, the cat CT is unresponsive to sucrose (Cohen *et al.*, 1955), contains no S neurons (Robinson, 1988), and cats cannot behaviorally discriminate sucrose from water (Carpenter, 1956). Yet, the cat CT, perhaps alternately, responds to L-amino acids (Boudreau *et al.*, 1975): cat amino-acid sensitive GG neurons respond to L-proline, L-lysine and L-histidine, which the cats prefer and may encounter with their carnivorous habits (White and Boudreau, 1975). Dog amino-acid sensitive GG units, as in cat the most common of those with receptive fields on the tip of the tongue, also respond to sugars (Boudreau *et al.* 1985). This carnivore, amino-acid sensing taste may utilize T1R1–T1R3, a heterodimeric GPCR, candidate amino-acid TR described for rodents (Li *et al.*, 2002). In humans, T1R1–T1R3 may be reduced to detecting glutamate (*umami*) taste (Li *et al.*, 2002; Nelson *et al.*, 2002).

According to Shi and Zhang (2006), T1R sweet receptors evolved more slowly than T2R, the candidate GPCR bitter receptors (cf., below); still, species variation in T1R structure approaches the variability associated with lineage-specific “environmental response” enzymes (Berenbaum, 2002; Hettinger *et al.*, 2007). Evolution equips sweet receptors with sufficient flexibility to accommodate the energy needs in the diverse habitats of many different species. In this context, it is not surprising that our closest relative, the chimpanzee, with a genomic DNA sequence similarity of 99% (nonpolymorphic) to the human (Kehrer-Sawatzki and Cooper, 2007), has CT S neuron sensitivities that appear identical to human sweet taste (Hellekant *et al.* 1997b, 1998). T1Rs are also located further within the alimentary canal in the gut, where they contribute to physiological responses (Dyer *et al.* 2005; Margolskee *et al.* 2007) that may, in the long term, influence sweet-stimulus intake.

4. 2. Ionic Tastes

Human tastes exclusively tied to ionic substances are salty and sour, but there are also bitter ionic substances. Tastes of many salts, such as KCl, are dipartite (salty and bitter), and other salts, such as CaCl₂, may be purely bitter (van der Klaauw and Smith, 1995). Tastes of ionic stimuli in other species may also have multiple qualities. Behavioral generalizations by rodents

identify 3 ionic tastes: one uniquely identified with sodium/lithium cations, the others more loosely identified with titratable acidity, which helps explain the greater effect of organic than inorganic acids at the same pH, or non-sodium inorganic bitter salts (Frank and Nowlis, 1989; Fig. 5).

Na^+/Li^+ cations are associated with neural activity in CT N neurons in rodents, sheep and goats; and multiple electrolytes and/or titratable acidity are associated with CT E neuron (acid unit) activity in rodents, cats, dogs and goats (Frank *et al.*, 1988; Nagai *et al.*, 1988; Boudreau *et al.*, 1985; Lundy and Contreras, 1999; Breza *et al.*, 2007). Like S neurons (Robinson, 1988), given species' or inbred mouse strains' peripheral systems dealing with ionic stimuli may be present or absent (Pfaffmann, 1955; Contreras *et al.*, 1985; Gannon and Contreras, 1995) and have species- and strain-dependent stimulus chemistries. This diversity, an ethological and genomic blessing important to recognize, may be a curse for identifying uniformities in taste coding strategies in many different species. Study of one species in depth has advantages, but that species' niche, biology and behavioral capacities must be considered.

4. 2.1. NaCl—The specificity of rodent CT N neurons (Fig. 4) coupled with a quite specific behavior toward sodium salts (Fig. 5) suggests that N neurons transmit essential information about sodium ions in food and water from taste bud to brain. The specificity with which a taste system detects Na^+ is not fixed across species and is perhaps associated with dietary niche. Rodents: rat, hamster, gerbil, some inbred mouse strains; and goats and sheep (Boudreau *et al.*, 1985; Nagai *et al.*, 1988; Frank *et al.*, 1988; Somenarin *et al.*, 1992) have a prominent salt-sensing system that is quite specific for Na^+ . However, neither rabbit (Beidler *et al.*, 1955; Contreras *et al.*, 1985) nor cat or dog (Boudreau *et al.*, 1985), nor pig or marmoset (Danilova *et al.*, 1999; Danilova *et al.* 2002; Danilova and Hellekant, 2004), nor rhesus monkey (Hellekant *et al.*, 1997a), chimpanzee (Hellekant *et al.*, 1997b) or human (Frank *et al.*, 2000; Ossbaard and Smith, 1995) have a taste system so focused on detection of Na^+ . The human salty taste, epitomized by the taste of NaCl, detects a strong saltiness in NaCl but a very weak saltiness in non-halide sodium salts (McBurney and Shick, 1971; Murphy *et al.*, 1981; Schiffman *et al.*, 1980; van der Klaauw and Smith, 1995; Frank *et al.*, 2001). Most non-sodium salts that are salty are also bitter to humans.

While maturing from fetus to lamb, sheep (*Ovis aries*), which have ~5 taste buds per fungiform papilla as do rhesus monkeys and humans (Bradley *et al.*, 1985; Cheng and Robinson, 1991; Just *et al.*, 2004), exhibit a concurrent increase in CT NaCl response and in the number of NaCl-best CT nerve fibers with small receptive fields (Mistretta *et al.*, 1988; Nagai *et al.*, 1988). The ontogenetic increase in small receptive fields is specific to NaCl-specialists; there are no changes in the CT electrolyte generalists' consistently larger receptive fields. The sheep's size provides an experimental advantage for fetal-nerve recording and gustatory receptive field mapping (Mistretta and Bradley, 1983; Hill and Mistretta, 1990). Postnatal development of CT NaCl responses and NaCl-best neurons in the altricial rat (Hill *et al.*, 1982) parallel precocial sheep, but there is neither an equivalent increase in sheep GL NaCl responses (Mistretta and Bradley, 1983) nor a postnatal developmental increase in the early substantial hamster CT NaCl responses (Hill, 1988). The sheep GL results are consistent with the scarcity of Na^+ -specialists and GL NaCl-response insusceptibility to amiloride in rat. And, responses of CT Na^+ -specialists, blocked by amiloride in hamster and rat, may appear ontogenetically earlier in hamsters, a desert rodent with relatively accelerated prenatal lingual development (Whitehead and Kachele, 1994). Sheep CT salt responses have not been tested with amiloride.

Rodents find the tastes of Na^+ salts to be quite distinct from NH_4^+ and K^+ salts (Fig. 5), unless the tongue is treated with amiloride, which blocks the epithelial Na^+ channel ENaC (Fig. 6). Rats do not become taste-blind for NaCl after amiloride treatment; NaCl is detected, but is no

longer distinguished from non- Na^+/Li^+ salts (Hill *et al.*, 1990), which hamsters behaviorally cross-generalize with bitter quinine (Fig. 5, Frank and Nowlis, 1989). After amiloride treatment, NaCl, KCl and NH_4Cl have a similar taste but remain discriminable from HCl (Fig. 6); and, unlike after CT section, rats detect NH_4Cl at normal concentrations (Geran and Spector, 2007).

Loss of Na^+ -specific taste behavior (Fig. 6) with amiloride treatment is associated with a specific reduction in responsiveness of CT N neurons in rats and hamsters (Ninomiya and Funakoshi, 1988; Fig. 7; Hettinger and Frank, 1990; Table 1, col. 8). NaCl responses of the GSP branch of CN VII are also blocked by amiloride (Sollars and Hill, 1998), but responses of the lingual branch of CN IX, the GL, are not blocked (Formaker and Hill, 1991; Kitada *et al.*, 1998). Thus, the means for the specific identification of sodium salts by rodents resides in facial nerve taste afferents; the GL is not involved (Spector and Grill, 1992; Markison *et al.*, 1995). Na^+ -specific N and electrolyte-generalist E neurons appear to develop (Hill and Bour, 1985) and recover from injury separately to form consistent connections with distinct targets in lingual taste buds, with N neurons establishing an exclusive interaction with TRCs expressing ENaC (Yasumatsu *et al.*, 2003; Shigemura *et al.*, 2005) and participating in distinct central brainstem circuits (Scott and Giza, 1990; Boughter and Smith, 1998).

Like “sweet”, a specific Na^+ sensing system, perhaps simply a CN VII taste-bud associated ENaC, evolved in response to rodent ecological necessity but likely degraded without selective pressure, as did the *Tas1R2* gene coding for the sugar receptor in cats (Li *et al.*, 2006), in other species like ours.

4. 2.2. Other Electrolytes—CT electrolyte-generalist E neurons, also known as acid units because of a strong response to acids (Fig. 7), are found in many species and have shorter response latencies than other GG neurons, suggesting larger fiber diameters (Boudreau *et al.*, 1985). Compared to CT S and N neurons, the role of CT E neurons in taste quality identification is not well understood (cf., section 3). Besides a response to titratable acidity in multiple species (Boudreau *et al.*, 1985; Sato *et al.*, 1975; Danilova *et al.*, 2002) that is curiously rare in chimpanzee (Hellekant *et al.*, 1997b), E neurons respond to salts and ionic bitter stimuli such as quinine HCl (Frank *et al.*, 2004). E afferent salt responses, in contrast to N specialist, are amiloride-insensitive (Fig. 7, Table 1, col. 8), yielding a residual NaCl response in rodent CT (Formaker and Hill, 1988; Hettinger and Frank, 1990; Ninomiya and Funakoshi, 1988; Yasumatsu *et al.*, 2003). Responses to NaCl and other halide salts in acid-sensitive CT E neurons appear to be restricted to rodents and goats (Boudreau *et al.*, 1985).

In rat, hamster and mouse, the salt stimulus chemistry of generalist E neurons is consistent with the indistinguishable behavioral responses towards Na^+ , Li^+ and K^+ salts after N neurons are blocked by amiloride (Fig. 6). This residual rat salt response is less cation specific, which resembles the stimulus chemistry of the human salty taste (Hettinger and Frank, 1992). Psychophysical data indicate that human saltiness may be unaffected by amiloride (Ossebaard and Smith, 1995); but, chlorhexidine, a bis-biguanide antiseptic, blocks salty, including the salty component of KCl, which, like other non- Na^+ salts, is salty-bitter to humans (Frank *et al.*, 2001; Breslin and Sharp, 2001). After blocking ENaC in engineered mice that do not express TRPV1, tonic CT responses to salts were eliminated (Lyll *et al.*, 2004); but remaining early transient responses may be sufficient to support wild-type, ENaC-independent, salt detection (Treesukosol *et al.*, 2007). TRPV1 is a nonspecific cation channel activated by heating to $>42^\circ\text{C}$; its chemical agonists include chemesthetic ligands such as the vanilloid capsaicin (Bandell *et al.*, 2007). Perhaps the tonic epoch of the CT salt response is partly a non-adapting, non-specific salt chemesthesis.

E neurons are more common in rat than hamster. One-half to two-thirds of rat CT neurons responsive to ionic stimuli are electrolyte generalists; whereas, just one-third of hamster CT neurons responsive to ionic stimuli are generalists (Contreras and Lundy, 2000; Frank *et al.*, 1983; Ninomiya and Funakoshi, 1988; Table 1, col. 1). The experimental focus on the rat, with its preponderance of CT electrolyte generalists, most of which respond to NaCl, contributed to the resilience of the pattern theory of taste quality coding (cf., section 2). Bitter salts like CaCl_2 and MgSO_4 , bitter quinine, and sour mineral and organic acids are also effective E-neuron stimuli. Neither the bitter nor sour stimuli cross-generalize with NaCl, pre- or post-amiloride (Fig. 4 and Fig 5). It is yet to be determined exactly how multiple E-neuron subtypes may be involved in taste quality coding (Lundy & Contreras, Breza *et al.*, 2007).

Hamster CT E neurons respond weakly to ionic bitter stimuli that are perceived as extremely bitter to people. CT responses to 1 mM quinine HCl and 3 mM denatonium benzoate are equally small and hamster behavioral thresholds, 0.3 mM and 1 mM respectively, are far above the human perceptual thresholds of 10 μM quinine and especially 10 nM denatonium (Frank *et al.*, 2004). Hamster-human threshold disparity factors for quinine: $0.3/0.01 = 30$ and denatonium: $1.0/0.00001 = 100,000$ compare the dissimilar sensitivity measures of intake in rodents to perceptual judgments in humans. Together, the quinine-denatonium threshold differences yield a *disparity ratio* of 3000. Two-compound *disparity ratios* derived from the odds-ratio concept and calculated from diagonals of 2×2 matrixes of detection thresholds, control for species-related measurement biases (Frank and Hettinger, 2005). The great difference in sensitivity makes it unlikely orthologous receptors detect denatonium in the two species. Hamsters readily “ingest a nut flavoured” with denatonium, “the most bitter substance known to man” (Wong, 1994). Nor is guinea pig consumption of feed pellets containing 1% denatonium disrupted (Nolte *et al.*, 1994). Rhesus monkey CT and GL nerves are insensitive to 100 μM denatonium (Hellekant *et al.*, 1997a); yet CT electrolyte-sensitive generalists of our closest genomic relative, the chimpanzee, strongly respond to 1 μM denatonium (Hellekant *et al.*, 1997b).

Rodent CT nerves are responsive to ionic bitter stimuli near behavioral threshold; in fact, the neural and aversion thresholds often fall within a half-log step of each other (Fig. 8; Frank, 1973; Contreras *et al.*, 1995; Coldwell and Tordorf, 1996; Inoue and Tordorf, 1998; Frank *et al.* 2004; Mueller *et al.*, 2005; Boughter *et al.*, 2005; Hisatsune *et al.*, 2007; Spector and Kopka, 2002). However, as pointed out in the next section, it is a different story for non-ionic bitter stimuli (Fig. 8; Boughter *et al.*, 2005; Frank *et al.* 2004; Hettinger *et al.*, 2007; Hisatsune *et al.*, 2007), which are more effective when applied to posterior oral regions within receptive fields of the glossopharyngeal nerve (Geran and Travers, 2006).

Rodent GL, electrolyte-sensitive, generalists are more responsive to acidity than CT E neurons and, unlike the CT, there are many GL specialists that are most-responsive to quinine HCl (Fig. 1A; Frank, 1991; Hanamori *et al.*, 1988). Yet, rodent GL nerves do not respond to lower concentrations of ionic quinine; rather the GL responds more strongly to high levels like 10 to 30 mM quinine than does the CT (Frank *et al.*, 1983; Hanamori *et al.* 1988; Danilova and Hellekant, 2003; Hisatsune *et al.*, 2007). Also, there are few GL N specialists (Frank, 1991; Hanamori *et al.*, 1988) and, predictably, responses of electrolyte-sensing afferent fibers in the rat GL nerve are unsuceptible to amiloride (Formaker and Hill, 1991; Kitada *et al.*, 1998). As noted above (cf., section 3), GL activation may assist behavioral discriminations between acids and ionic bitter stimuli made by rats via activation of GG acid-generalists and quinine-generalists (Breza *et al.*, 2007). However, the aversive non-ionic bitter stimuli caffeine and sucrose octaacetate (SOA) fail to activate the hamster CT (Frank *et al.*, 2004) and threshold CT activation by non-ionic cycloheximide is more than 10,000 times higher than the hamster aversion threshold (Hettinger *et al.*, 2007).

4. 3. Non-ionic Bitter Tastes

The especially great species diversity for high-affinity “bitter” tastes (Hettinger *et al.*, 2007) reflects an extremely rapid T2R evolution (Shi *et al.*, 2003; Shi and Zhang, 2006; Chandrashekar *et al.*, 2006). For example, humans are 100,000 times more sensitive to ionic denatonium and hamsters are 3000 times more sensitive to non-ionic cycloheximide. The overall human-hamster, denatonium-cycloheximide disparity ratio of 300,000,000, suggests that distinct receptors for ionic and non-ionic bitter compounds for the two species may not be exceptions but the rule. Disparity indices are much larger when one species is more sensitive to one, and the other species more sensitive to the other compound. The mouse T2R receptor specific for cycloheximide, T2R5, is coded for by a gene in a 5-member clade on chromosome 6; there is an orthologous rat clade, but there are no human or rabbit orthologues. The 35 rodent, compared to 25 human, functional T2Rs (Behrens and Meyerhof, 2006) allow rodents to sense “bitters” our species cannot detect, genetic volatility that makes it difficult to define bitter taste across species (Hettinger *et al.*, 2007).

Exact roles of T2R receptors in bitter taste sensation are unknown. The hamster CT nerve responds barely at all to the non-ionic bitter stimuli caffeine, SOA, *n*-propylthiouracil, and cycloheximide (Fig. 8) even though hamsters avoid each compound (Frank *et al.*, 2004; Hettinger *et al.*, 2007). B6 mouse GL nerves respond to lingual stimulation with 100 μ M cycloheximide (Danilova and Hellekant, 2003) and a subset of rat “bitter-best” brainstem NST neurons respond well to 10 μ M cycloheximide applied to the GL receptive field (Geran and Travers, 2006). But, the specific adverse perceptual qualities associated with cycloheximide and other non-ionic bitter stimuli in rodents are unknown.

In hamsters, neither cycloheximide (Hettinger *et al.*, 2007) nor caffeine or SOA behaviorally generalize to quinine HCl or denatonium benzoate (Fig. 9; Frank *et al.*, 2004), 2 ionic bitters that taste quite similar to humans and rodents (Spector and Kopla, 2002). Unlike humans, who may perceive a unitary bitter sensation, rodents likely experience multiple distinct adverse percepts when sampling bitter stimuli and use information other than CT neural activity, to meticulously avoid stimuli that humans can hardly detect.

Compared to “sweet” T1R sensing, T2R-based systems evolved much more rapidly (Shi and Zhang, 2006). Differences between rodents and humans likely emerged in response to ancient prehistoric ecological niches, which contained dangers from toxins generated by other species, and subsequent gene duplication (Hettinger *et al.*, 2007). Cycloheximide, for example, detected by a rodent-specific high-affinity T2R (Chandrashekar *et al.*, 2000), is a natural lethal antibiotic produced by microbes living in soil and possibly a grave danger to fossorial rodents (Hettinger *et al.*, 2007). T2R lineage specificity exaggerates the difficulty in identifying T2R, compared to T1R, ligands. CN VII fields, where T1Rs, specific Na⁺ receptors and aversive-electrolyte receptors are located, focus on taste discrimination more than CN IX fields, where T2Rs predominate and reflexes are initiated (Adler *et al.*, 2000; St John and Spector, 1998; King *et al.*, 2000). A posterior lingual T2R locus is consistent with CN IX brainstem, projection sites for high-affinity, cycloheximide neural activity (Geran and Travers, 2006); activity that elicits sensations quite unlike quinine (Frank *et al.*, 2003) as well as protective reflexes (King *et al.*, 2000) in rodents. Additional complications are gut T2R receptors (Rozengurt, 2006), which serve distinctly different functions than taste sensation, but possibly contribute to intake behavior subsequent to ingestion.

5. Contexts, Needs and Experiences Matter

Gustatory coding is dynamic, capable of detecting transient changes in a chemical milieu. Taste stimuli in food and drink are introduced into a complex oral chemical context and tastes are perceived transiently above an ambient salinity (Bartoshuk, 1974). A constant component in

the oral environment is saliva, which is itself a variable formulation (Schneyer *et al.*, 1972); and single-stimulus, *self-adaptation*, or multiple stimulus, *cross-adaptation*, control our perceptual experiences. Evaluation of peripheral taste information, which contributes to decisions to ingest or reject, adjusts to nutritional needs via receptivity to systemic signals (Contreras and Frank, 1979) and peripheral taste signaling adjusts to dangerous substances present in available sources of food and drink (Formaker and Frank, 1996). Neural activity carried to the brain by gustatory afferent neurons is malleable, reflecting a variable oral milieu, internal needs and nutrient sources. In addition, experiencing illness after tasting modifies preferences for taste stimuli centrally, via taste aversion learning (Yamamoto, 1998).

5. 1. Oral Context, Saliva and Adaptation

Taste stimuli adapt in time, with saliva providing a background “tasteless” context for identification of stimuli introduced into the mouth. Prominent salivary ions: Na^+ , K^+ and Cl^- , levels depending on species (Rehnberg *et al.*, 1992), salivary gland and flow rate (Schneyer *et al.*, 1972), are important gustatory stimuli (cf., section 2, section 3 and section 4). The salty prototype NaCl is identifiable as such only when it is above ambient salinity, about 10 mM in humans (McBurney and Pfaffmann, 1963); below this NaCl tastes slightly bitter, like water (Bartoshuk, 1974). Rats also find NaCl salt-like above but water-like below salivary levels (Morrison, 1969). Adapting to higher levels of NaCl can reduce the saltiness of many electrolytes to nil. After adapting to 100 mM NaCl to eliminate *salty* taste, KCl, NH_4Cl and citric acid become increasingly bitter to humans (Smith and McBurney, 1969; Smith and van der Klaauw, 1995). In the mouth, tastes of newly introduced electrolytes wax and then wane to reduce to water-like/bitter with adaptation. Bitter “aftertastes” may reflect remaining activity of rodent CT electrolyte generalists (cf., amiloride effect on salt taste, section 4) or disinhibition of specific TRs, as suggested for human sweet aftertastes (Galindo-Cuspinera *et al.*, 2006).

Human taste adapts exponentially, approaching zero with a 30-sec half-life (Gent and McBurney, 1978). In contrast, there is hardly complete adaptation of the rat whole-nerve CT NaCl response. Neural spiking of rat specialist N and generalist E nerve fibers are combined in the whole-nerve (Frank *et al.*, 1983; Lundy and Contreras, 1999). Following a few-second transient response, a high level of tonic neural activity continues indefinitely (Fig. 1B, Smith *et al.*, 1978; cf., section 2) but may no longer elicit a taste. Like repeat stimulation with NaCl without rinsing, inorganic Na^+ and Li^+ salts, which activate N and E neurons, cross-adapt only the short transient epoch of rodent CT response (Smith and Frank, 1972; Rehnberg *et al.*, 1989). However, CT transient responses to NH_4^+ , K^+ , Ca^{++} and Mg^{++} inorganic salts, which primarily activate E neurons, cross-adapt more individually. There is a myriad of unexplored specific dynamic phenomena deciding which information goes to the brain via afferent neurons.

Hamster CT N-neuron responses to NaCl and sodium acetate (Fig. 10) and rat CT N-neuron responses to NaCl and monosodium glutamate (MSG) dynamically and asymmetrically cross-adapt (Rehnberg *et al.*, 1993; Breza *et al.*, 2007). Adapted responses transiently increase when chloride replaces the organic anion, suggestive of Beidler’s cation–anion, excitatory–inhibitory, formalism (Beidler, 1967; 1971), possibly more dramatic in E neurons, which are more sensitive to anion size (Table 1). Dynamic CT responses to change in anion may be reflected in transient salt tastes, given humans can detect a 1-Hz 5% transient experimental modulation in 100 mM NaCl concentration (McBurney, 1976). Behavioral studies are needed to identify sensations associated with tonic responses and dynamic CT N-neuron and E-neuron responses to stimulus change. Interestingly, toads evaluate electrolytes in water sources that they use to rehydrate via “seat-patch” receptors (Hillyard *et al.*, 2004).

Adaptation, expected for salts with natural saliva present, is seen in the CT nerve in behaving rats (Matsuo and Yamamoto, 1990) or after rinsing the tongue with artificial saliva in anesthetized hamsters (Rehnberg *et al.*, 1992). Natural saliva also enhances rat CT responses to sweet sugar (Matsuo and Yamamoto, 1990) and suppresses hamster CT responses to bitter quinine (Rehnberg *et al.*, 1992). The enhanced sucrose responses may be partly mediated by the high pH of saliva, which may simultaneously depress quinine responses by conversion of stimulatory quininium (quinine H⁺) cation to the less stimulatory non-ionic free base (Matsuo and Yamamoto, 1992; Rehnberg *et al.*, 1992). But other more interactive biological processing may be involved.

Hamsters do not behaviorally recognize 1 mM quinine when mixed with 100 mM NaCl (Frank *et al.*, 2003). And, when the tongue is rinsed with water, hamster CT responses to 30 mM quinine HCl are reduced to zero in mixtures containing 50 mM or more NaCl (Formaker and Frank, 1996). In a saliva adapted context, low concentrations of quinine in water may be detected by a transient inhibition of neural activity (Rehnberg *et al.*, 1992). The mechanism by which NaCl inhibits quinine responses of CT electrolyte generalists is unknown but its correlation with behavior suggests it occurs in all contributing taste bud populations. Concurrent studies of gustatory adaptation behaviorally and in Na⁺ specialists and electrolyte generalists under naturally occurring conditions are needed to appreciate the significance of salt-bitter electrolyte interactions in mammalian taste quality coding. In people, NaCl may be used as a condiment to “enhance flavour by suppressing bitterness” (Breslin and Beauchamp, 1997).

5. 2. Needs and Experiences, Malleable Specialist Coding

The coding of taste stimuli by CT specialists, signs for nutritional sources, is modifiable (Table 1, col. 6 & 9). Stimulus salience is altered by dampening responses of Na⁺-specialists, allowing induced salt appetite to reestablish electrolyte homeostasis in rats (Contreras and Frank, 1979; Contreras and Lundy, 2000). Similarly, responses of hamster sucrose-best specialists to sucrose and other sweeteners are muted when stimulus contamination makes ingestion for calories dangerous (Formaker and Frank, 1996; Frank, 2000). Nevertheless, stimulus quality, the Na⁺-salt and sweet tastes, are retained; in fact S afferent neurons continue with their 1.4 Hz “sweet rhythm” (Frank *et al.*, 2005).

5. 2.1. Sodium Appetite—Na⁺ is an essential nutrient for life, and its level in the body is tightly regulated. Salt appetite, the behavioral manifestation of a negative Na⁺ balance, is characterized by a switch from avoidance of concentrated sodium salt to avid ingestion in rats (Contreras and Frank, 1979; Lundy Jr. *et al.*, 2003), but it is difficult to demonstrate Na⁺ appetite in desert rodents like hamsters (Fitts *et al.*, 1983). The rats’ change in acceptance is accompanied by a specific inhibition in peripheral neural responses to solutions containing Na⁺. With 10 days of Na⁺ deprivation, the CT NaCl response is reduced compared to controls due to a reduction in response rates of Na⁺-salt specialist neurons (Contreras and Frank, 1979; Table 1, col. 9; Fig. 11), the same neurons blocked by amiloride (Table 1, col. 8; cf., section 3).

A similar reduction in NaCl-evoked activity follows removal of the adrenal glands, which eliminates secretion of the hormone aldosterone responsible for Na⁺ re-absorption (Contreras *et al.*, 1984; Kosten and Contreras, 1985). Importantly, NaCl responses of electrolyte generalist are unaffected by Na⁺ deprivation and amiloride. Na⁺-salt specialists carry a specific message that is critical for elaboration of sodium appetite (Bernstein and Hennessy, 1987; Contreras and Lundy, 2000; McCutcheon, 1991), which is eliminated by blocking of Na⁺-specialist activity with amiloride and impaired with CT section leaving the GSP intact (Breslin *et al.*, 1993). Plasticity in what is transmitted to the brain by neurons in CN VII allows taste signals to play a critical role in maintenance of systemic Na⁺ balance (Daniels and Fluharty, 2004).

Precise physiological changes underlying reduced reactions of Na⁺-specialist neurons after Na⁺ deprivation or adrenalectomy remain speculative. Deprivation reduces salivary Na⁺ and increases aldosterone, while adrenalectomy increases salivary Na⁺ in the absence of aldosterone (Contreras and Frank 1979; Contreras and Catalanotto, 1980); but levels of 2 other hormones, angiotensin II and adrenocorticotropin hormone, increase after either manipulation. One hypothesis is that Na⁺ restriction leads to reduced numbers of conducting ENaC channels in cell membranes of TRC innervated by Na⁺-specialists (Contreras *et al.*, 1984; Stewart *et al.*, 1995). This reduction may be achieved through hormonal or genomic regulation of ENaC expression (Herness, 1992; Lundy Jr., 1998; Yasumatsu *et al.*, 2003). Genomic control may also allow rats born and raised on low Na⁺ to recover amiloride susceptibility when Na⁺ is added to the diet (Pittman and Contreras, 2002); greater Na⁺-specialist efficacy may be induced by the excess Na⁺.

5. 2.2. Mixture Suppression, Sucrose-Quinine Mixes—Sucrose responses in hamster CT sucrose-best specialists are diminished when the sucrose is mixed with quinine HCl (Formaker *et al.*, 1997; Table 1, col. 6). Ten mM quinine essentially eliminates CT responses to 100 mM sucrose (Fig. 12). The suppression is concentration-dependent, and sucrose response decrements in the S specialists are quantitatively matched by quinine response increments in electrolyte-sensitive generalists (Frank *et al.*, 2005; Table 1, col. 2; cf., section 3). In fact, S specialist responses to sucrose mixed with electrolytes are inversely related, $r = -0.96$, $p < .01$, to E-generalist neural activity elicited by stimulation with the electrolyte series: HCl > NH₄Cl > CaCl₂ > NaCl (Hyman and Frank, 1980). The mechanism of the inhibition is unknown, but it is possibly involve a specific intercellular interaction between electrolyte - activated TRCs and sucrose-activated TRCs in the taste bud, before transmission to the brain by CN VII (c.f. section 6.2 below). Quinine directly inhibits the non-selective, heat-sensitive, cation channel TRPM5 (Talavera *et al.*, 2005; Talavera *et al.*, 2008), which plays a ubiquitous role in post-TR taste transduction (Zhang *et al.*, 2003), predictive of a non-specific effects on TR function.

Susceptibility to inhibition of sucrose responses by gurmarin, a polypeptide isolated from *Gymnema sylvestre*, reveals subtypes of mouse CT quality-specific S neurons (Ninomiya *et al.*, 1999). The inhibition is inbred-strain, CT specific, and not associated with T1R polymorphisms (Ninomiya *et al.*, 2005; 1997). Gurmarin inhibition also suppresses licking of sweetener-quinine mixtures (Murata *et al.*, 2003). The peptide may activate an indirect sweet-inhibition mechanism such as the one suggested above for bitter substances.

Sucrose and quinine are each behaviorally recognized by hamsters in a mix of 100 mM sucrose with 1 mM quinine (Frank *et al.*, 2003), a level of contamination by the bitter quinine where both CT S neural activity and the sweet rhythm of sucrose are preserved (Fig. 12; Frank *et al.*, 2005). With sufficient neural signals, hamsters analyze sucrose-quinine as they analyze sucrose-NaCl mixtures, which rats also behaviorally analyze when given various levels of NaCl and sucrose (Smith and Theodore, 1984). The CN VII carbohydrate-detecting S neurons are specifically inhibited, like invertebrate gustatory signals (Dethier and Bowdan, 1989; Li *et al.*, 2001), when sources of calories contain potentially toxic compounds. The adjusted salience reduces intake, but the sweet taste quality is unchanged.

Substances involved in energy homeostasis, such as blood glucose, insulin, and leptin, also reduce sweet-stimulus intake (Oetting and VanderWeele, 1985; Giza and Scott, 1987a; Campfield *et al.*, 1996). The influence of circulating factors is short-term, associated with postprandial satiety, mediated by central mechanisms, and can be sweet-quality specific (Giza and Scott, 1987b; Chavez *et al.*, 1996; Moran *et al.*, 2006). Intravenous administration of glucose or insulin specifically suppresses the response of NTS brainstem neurons to lingual application of sugars (Giza and Scott, 1987b; Giza *et al.*, 1992). However, leptin may act

peripherally on TRCs to suppress “sweet” sucrose and saccharin intake. Leptin-receptor mRNA is found in mouse fungiform and circumvallate taste buds; leptin application hyperpolarizes TRCs isolated from the circumvallate papillae and specifically suppresses CT and GL sucrose and saccharin responses (Kawai *et al.*, 2000; Shigemura *et al.*, 2004). Sucrose and saccharin activate CT S specialists. Responses to stimuli that activate N specialists and E generalists were not suppressed by leptin application. Gustatory stimulus saliency appears to be adjusted to accommodate the safety/control of caloric intake as well as sodium appetite.

5. 2.3. Conditioned Taste Aversion Learning—Painful gastrointestinal consequences of an ingested taste stimulus induce a conditioned taste aversion (CTA) that reduces subsequent intake (Bures *et al.*, 1998). CTAs are learned in natural settings by ingesting a taste stimulus mixed with a tasteless toxin or by being “fooled by taste” into self-administering a toxin such as LiCl (Contreras and Lundy, 2000; Nachman 1963; 1973). Rats initially drink solutions of LiCl, which like NaCl activates CT Na⁺ specialists (Fig. 7), but later avoid both salts via CTA generalization. Hamsters’ increased avoidance of cycloheximide, a deadly toxin, after one exposure may likewise involve a CTA and/or genomic induction of T2Rs above constitutive levels (Hettinger *et al.*, 2007).

CTAs are reversible, stimulus-quality specific, and constructed by the central nervous system (Yamamoto, 1998). The learned aversions reduce stimulus intake by altering valance (preference), without changing stimulus quality: the environmental cue for CTA learning (Nowlis, 1974), and can last quite a long time before extinguishing. The plasticity in valance is used experimentally to identify stimuli that taste alike by measuring behavioral generalizations, which have provided important data for taste quality coding studies (cf., section 3). A CTA to “sweet” sucrose does not generalize to aversive stimuli; but rather generalizes to other sweet compounds, those that activate sucrose-best specialists (Nowlis *et al.*, 1980; MacKinnon *et al.*, 1999; Frank and Nowlis, 1989; Fig. 3). “Sweet” becomes less attractive.

6. Taste Quality Codes

Unexpected non-specificity in the very first taste nerve recordings led to the idea that “across fiber patterns” may exclusively code taste qualities (Pfaffmann, 1941), a concept that persevered in spite of many, subsequent neural recordings demonstrating species-dependent specificity that matched behavioral simplicity (e.g., Hellekant *et al.* 1997a, Ninomiya and Funakoshi, 1988, Contreras and Lundy, 2000; Frank, 2000). With discovery and characterization of TRs with powerful genetic techniques at the beginning of this century, a critical reassessment was possible. Genetic engineers were able to create mice with specific defects in taste receptors that revealed an initial taste quality coding of “elegant simplicity,” supporting “labeled line models” (Chandrasekar *et al.* 2006). In fact, mice were created to permit specific activation of T1R2-containing TRC by an unnatural compound, which the mice preferred as if it were sugar (Zhao *et al.*, 2003): a modern feat, reminiscent of observations that led to Johannes Müller’s 1826 law of “the specific energies of the senses” (Riese and Arrington, 1963), but for sweet preference.

In Table 2, effects of “knocking out” rodent candidate TR are compared to the stimulus chemistries of rodent CT neurons types. Not included are T1R and T2R in posterior tongue fields innervated by the GL, CN IX, (Frank, 1991; King *et al.*, 2000) or in the throat (Hanamori & Smith, 1991; Bradley, 2000) or gut (Rozengurt 2006; Dyer *et al.*, 2005; Margolskee *et al.*, 2007) innervated by CN X. In rodents, CN IX taste-bud containing receptive fields are more involved in ingesting/rejecting via brain-stem motor systems (Travers and Travers, 2005), and CN X receptive fields more involved in protecting the airway (Bradley, 2000) or participating in metabolic pathways in the gut, (Margolskee *et al.*, 2007) than sensing taste-stimulus quality.

Although the CT assumes some GL reflexive functions when cross-wired to posterior fields (King *et al.*, 2008), it is not yet known that the GL can take over the well-documented CT-mediated discrimination of taste quality (St John and Spector, 1998; Geran *et al.*, 2002).

Activated taste buds initiate coded messages in the form of neural impulses that are carried by afferent taste neurons to the brain to mediate taste perceptions. However, it is becoming increasingly clear that the TRC to afferent nerve fiber link is not a simple relay. Understanding how codes are generated in TRC–afferent nerve circuits is now being experimentally addressed.

6. 1. Taste Receptor - CT Neuron/Behavior Concordance-Discordance

Neural recordings and behavioral deficits showed that sets of G-protein coupled TRs (Adler *et al.*, 2000; Li *et al.*, 2002; Nelson *et al.*, 2001, 2002; 2005), a PDK2 TRP-channel TR (Huang *et al.*, 2006), and, earlier, identified sets of amiloride-sensitive (Ninomiya and Funakoshi, 1988; Hettinger and Frank, 1990) and sugar-sensitive CN VII neurons (Frank, 1973; Nowlis and Frank, 1989; Hellekant *et al.*, 1998) were more or less consistent with labeled lines for distinct taste qualities having species-specific stimulus chemistries (Nowlis *et al.*, 1980; McBurney and Gent, 1979).

Molecular genetics provides compelling evidence that specific T1Rs, multiple T2Rs and a TRP channel segregate in sets of TRC dedicated to sweet, glutamate, bitter and sour taste (Chandrashekar *et al.* 2006). Electrophysiological and pharmacological evidence support dedicated CN VII channels for “sweet” and Na⁺ taste (Lundy and Contreras, 1999) but neural channels for sour, bitter or glutamate or fat taste are yet to be identified (Breza *et al.* 2007; Geran & Travers, 2006). TRC to CT neuron circuits should be consistent with categories of specialist CN VII neurons that are isomorphic with behaviorally identified taste qualities and the peripheral malleability associated with CT specialists noted above (cf., section 4).

The stimulus chemistry of the heterodimeric T1R2-R3 candidate “sweet receptor” (Nelson *et al.*, 2001), CT S specialist neurons (Table 2: col. 2), and behavioral generalizations to “sweet stimuli” (cf., section 3) are mostly concordant. However, CT S-neurons respond to glutamate (Formaker *et al.*, 2004; Breza *et al.*, 2007) and sucrose and MSG are behaviorally generalized. Furthermore, although molecular genetic information is unavailable, stimulus chemistry of the candidate “ENaC” associated receptor, CT Na⁺-specialists and behavioral generalizations (cf., section 3) are concordant (Table 2, col. 6). In this model, NaCl responses of electrolyte generalist CT neurons would not be involved in generation of the Na⁺-specific salt taste. Thus, the 2 CT neuron specialists may well be more or less dedicated circuits from specific TR-expressing TRC to the brain.

The mouse candidate glutamate (amino acid) receptor, heterodimeric T1R1-3, has distinct stimulus chemistry not recognizable in a single rodent CT neuron class (Table 2, col. 3). At 50 mM, most L amino acids, themselves or with 2.5 mM of the purine nucleotide IMP added, are effective T1R1-3 ligands; but, D enantiomers and sugars are ineffective (Nelson *et al.*, 2002). Rodents ascribe multiple taste qualities to MSG, in addition to the sucrose-like quality noted above (Yamamoto *et al.*, 1991; Heyer *et al.*, 2003; Delay *et al.*, 2007), even when N-neuron responses to Na⁺ are inhibited by amiloride (Table 2, col. 3). Glutamate-responsive, sugar-insensitive subsets of rat CT electrolyte generalist neurons are identified (Sako *et al.*, 2003; Formaker *et al.*, 2004; Breza *et al.*, 2007); but CT neurons, which have even lower affinities for L amino acids than GL neurons (Pritchard and Scott, 1982; Ninomiya *et al.* 1993), are unlikely to detect levels of essential amino acids found in nature. This contrasts with nM amino-acid-sensing, facial neurons of catfish (Kohbara *et al.*, 1992), who forage with their barbels in detritus, containing protein breakdown products, under water (Caprio *et al.*, 1993; Valentincic and Caprio, 1994). Interpretation of amino-acid T1R species specificity,

verified with mouse CT whole-nerve recordings (Li *et al.*, 2002; Nelson *et al.*, Zhao *et al.*, 2003), requires study of CT single neurons to detect distinct contributions of several neuron types to resolve apparent discordant T1R1-3, CT neuron and behavioral stimulus chemistries.

The stimulus chemistry of neither candidate T2R bitter nor PDK2 TRP-channel sour receptors (Nelson *et al.*, 2005; Huang *et al.*, 2006) are concordant with particular CT neuron types (Table 2: cols. 4 and 5). It is possible that TRCs specialized for sour or bitter converge onto CT generalists to signal dangerous compounds; however, this would leave distinct generalization patterns for bitter stimuli and acids unexplained (cf., Fig.4). Resolution may lie in subgroups of generalist neurons more specialized for ionic bitter stimuli or organic acids, as suggested by Breza *et al.*, 2007, and/or the contributions of other than CT neurons.

6. 2. Taste Bud to CT Neuron Circuits

Taste buds are now understood to be complex information processing units with possible dynamic communication among 3, structurally and functionally defined cell types (Finger, 2005; Finger *et al.*, 2005; Roper, 2006; Tomchik *et al.*, 2007; Kataoka *et al.*, 2008). Distinct sets of TRC may be dedicated to a particular “quality” by a cell-exclusive expression of taste receptors (Chadrashekar *et al.*, 2006). Yet, in the GL receptive field, functionally and histochemically distinct TRC, type II or III, either exclusively express molecular TRs, or exclusively make traditional vesicular synaptic contacts with afferent nerve fibers. How then may TRC activation be transferred unmodified to CT peripheral neuron (Yoshida *et al.*, 2006a)?

Recent evidence suggests type II TRC, containing all G-protein coupled TR, may communicate with other TRC or afferent GL neurons via pannexin hemichannels (Romanov *et al.* 2007; Huang *et al.*, 2007). A circuit like the latter but in the CT field, TRC_{II} → S-specialist afferent endings, would be concordant with a functional isomorphism between stimulus chemistries of heterodimeric T1R, CT-S neurons, and behavioral generalizations in rodents (Table 2). TRC_{III}, with traditional vesicular synapses onto taste afferents, specifically express the PDK2 channel associated with sour taste (Huang *et al.*, 2006; Katoaka *et al.*, 2008). However, perhaps, in part, because of multiple effects of H⁺ on general cell function (Lyall *et al.*, 2006; Huang *et al.*, 2006), no sour-specific CT neuron-type is yet identified (Table 2). In addition, at this time, neither the taste-bud cell types of action-potential producing TRC that transfer information to CT afferent endings (Yoshida *et al.*, 2006b), nor the TRC circuits (Tomchik *et al.*, 2007) that account for N specialist and E generalist stimulus chemistries (Breza *et al.*, 2007) are identified. Albeit expression of ENaC in mouse TRC occurs before CT Na⁺-specialist activity appears during taste-bud reconstitution (Shigemura *et al.*, 2005; Yasumatsu *et al.*, 2003) and a single nucleotide polymorphism in the α -subunit in ENaC is associated with mouse-strain differences in amiloride CT response inhibition (Shigemura *et al.*, 2008), localization of ENaC inhibition to a particular TRC type is tentative (Vandenbeuch *et al.*, 2008).

Although not genetically ablated, CT Na⁺-specialist, N-neuron responses are concordantly “knocked out” pharmacologically with an amiloride-block of ENaC (Table 2 col. 6), which disrupts Na⁺-specific rodent taste discrimination. However, genetically engineered mice lacking either of the 2 heterodimeric T1Rs demonstrate discordances with higher specificities than predicted by nerve recordings (Table 2, cols. 2 and 3) or behavior. S neurons respond to sucrose and glutamate, which are behaviorally cross-generalized, and glutamate also activates sucrose-insensitive E neurons. Perhaps S specialists have input from both heterodimeric T1Rs, and/or some E neurons may obtain input from the T1R1-3 heterodimer

The most serious discordance is attributable to rodent CT electrolyte-sensitive generalists. E-neuron stimulus chemistry is neither associated with specific tastes, behaviorally, nor linked

to specific candidate receptors (Table 2, cols. 4 and 5). Electrophysiological study of CT neuronal responses to natural and behaviorally characterized diverse bitter and sour stimuli, rather than damagingly high levels of chemical reagents, may serve to link subsets of E neurons to multiple behavioral discriminations of sour and bitter stimuli (Fig. 5 and Fig 9) and T2R, PDK2, or as yet unidentified chemosensory receptors (c.f., Liberles and Buck, 2006). But as of now, the T2R candidate bitter and PDK2 candidate sour receptors studied with genetically engineered mice, albeit concordant with behaviors, are discordant with known stimulus chemistries of CT E generalists, which, notably, all respond to NaCl (Breza *et al.*, 2007), with the tongue adapted to water not saliva.

6. 3. The Next Frontier

During the final quarter of the 20th century, before discovery of the TRs, ~1000 rodent olfactory chemoreceptors were discovered (Axel, 2005; Buck, 2005) and led to the realization that discrete biochemical reactions are the currency of chemosensory domains, which need no initial conversion from spectral domains as does vision (Bargmann, 2006; Frank, 2008). Genomic sequencing was followed by the ongoing candidate TR epoch (Chandrashekar *et al.*, 2006), which makes it possible to critically evaluate TRC to afferent neuron circuits that create the isomorphism between stimulus chemistries of CT-neurons and distinct-behaviors (Frank, 2000).

In evolutionary time, gustatory systems rapidly evolved receptors for sweet, and even more rapidly, receptors for bitter (Shi and Zhang, 2006; Shi *et al.*, 2003) in order to identify natural ligands as signs of nourishment or danger within a then species-dependent ecological niche. Within those diverse niches, real-life chemosensory stimuli are invariably complex mixtures with shifting component composition from which un-adapted new and significant components need to be identified.

A future epoch of discovery in gustatory science will address the impact of component sensory adaptation on recognition of compounds in natural taste mixtures, likely uncovering a taste coding strategy analogous to a dynamic odor coding (Goyert *et al.*, 2007, Marshall *et al.*, 2006; Frank, 2008). Thereafter, appreciation of the ephemeral detection of static sensory stimuli (Riggs *et al.*, 1953; Land, 1999), and the dynamic multitasking in central neural networks that form sensory perceptions (Katz, 2005; Fontanini *et al.* 2008) by “inference” (Ramachandran, 2005; Gawande, 2008) will forge a sea change in understanding of sensory system function.

6.4. Conclusion

Discovery of genes for TR candidate taste receptors at the turn of the century was a milestone for taste quality coding. No longer was it necessary to deduce “receptors” using correlations between stimulus chemistry and biology/behavior. With genomics, the natural history of taste receptors could be established, and with genetic engineering, effects of gene products on neuronal activity and sensation evaluated. It was already clear that the several taste-bud containing chemoreceptive fields participated in reflexes as well as sensation but it is now known that the very same chemosensory receptors serve metabolism, reflexes, and sensory perception. Stimulus chemistry associated with ecological niches and speciation were also already appreciated, but gene structures reveal that taste receptors evolved rapidly, especially rapidly for dangerous bitter stimuli. Although the taste-receptor structural and functional diversity, an ethological blessing, is a conundrum when trying to identify consistencies in taste coding, debate is no longer simply whether taste quality is a labeled line or pattern. Rather, debate is a multifaceted: which, how, where and why specific gustatory information is represented in species’ neural circuits?

Glossary

Abbreviations

BDNF	brain-derived neurotrophic factor
CN	cranial nerve
CT	chorda tympani nerve
CTA	conditioned taste aversion
ENaC	epithelial sodium channel
GG	geniculate ganglion
GL	glossopharyngeal nerve
GPCR	G-protein coupled receptor
GSP	greater superficial petrosal nerve
IMP	inosine 5'-monophosphate, a <i>umami</i> substance
MSG	monosodium glutamate
NST	nucleus of solitary tract
OR	olfactory receptor
SOA	sucrose octaacetate
T1R	candidate sweet/glutamate receptor
T2R	candidate bitter receptor
TR	taste receptor
TRC	taste-bud receptor cells
TRPM5	

non-specific transient receptor potential (TRP) cation channel

TRPV1

capsaicin-sensitive, TRP channel responsive to high temperature

Glossary

Adaptation

Adaptation is a decrease in response while continuously presenting a stimulus. Cross adaptation is a decrease in response to stimulus B after stimulus A has been presented.

S neuron

An S neuron is a CT nerve-fiber or GG cell with a sucrose-best response profile, a sweet stimulus specialist.

N neuron

An N neuron is a CT nerve-fiber or GG cell with an NaCl-best response profile, a Na⁺ specialist.

E neuron

An E neuron is a CT nerve-fiber or GG cell with an electrolyte-sensitive (also called acid-best) response profile, an electrolyte generalist.

Generalist

A generalist is a neuron that responds to stimuli with distinctly different taste qualities.

Response profile

A response profile determines the stimulus chemistry of an afferent neuron or behavioral generalizations of a species' across a battery of stimuli.

Specialist

A specialist is a neuron that responds to stimuli with similar taste quality.

Taste

“Taste” is used to mean *gustatory: related to taste perception*, like “taste bud” and TR. Neither are exclusively gustatory; nor are “taste” stimuli to which they respond or the “taste” nerves they activate.

Tuning

A specialist is finely tuned and a generalist is broadly tuned.

Acknowledgements

This review, supported by NIH NIDCD grants DC004099 and DC004849 (MEF), DC004785 (RJC), and DC006698 (RFL), is dedicated to the memory of Y Zotterman, C Pfaffmann and LM Beidler, who founded taste-nerve electrophysiology while establishing a multidisciplinary chemosensory specialty. We acknowledge the Pfaffmann memoir (1984) for inspiring the title and 2 anonymous reviewers for their insights, which are incorporated into this review.

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Figure 1. Electrophysiological recordings are obtained from single afferent neurons and whole afferent nerves

A. By teasing fascicles from a de-sheathed afferent nerve cut before it enters the brain, action potentials of single fibers are recorded. The example illustrated is from the rat glossopharyngeal nerve (Frank, 1991); stimuli were applied from arrow to end of record. **B.** The overlapping action potentials from hundreds of fibers in a whole nerve are rectified and integrated to monitor total neural activity. The example illustrated is from the hamster chorda tympani nerve (Hettinger and Frank, 1987); periods of application of NaCl and amiloride are designated by horizontal lines.

Figure 2. Specialist hamster chorda tympani neurons with sucrose-best response profiles transmit information to the brain about sweeteners

Mean ($\pm$ se) neural response rates to 0.1 M sucrose, 30 mM NaCl, 3 mM HCl, 1mM quinine HCl and control spontaneous neural spiking rates for 10 hamster chorda tympani (CT) S neurons are plotted. Note the high variation in sampled means. Data, $\frac{1}{4}$ th of a sample of 40 taste-responsive CT neurons, are from Frank *et al.*, 1988.

Figure 3. Hamsters' learned aversions to the other sweeteners saccharin and fructose generalize to 100 mM sucrose

Aversions learned to ionic stimuli do not generalize to sucrose. CT S-fibers respond to sweeteners but do not respond consistently to ionic stimuli and likely provide the “sweet” cue for hamsters. The CS groups learned aversions to 100 mM sucrose, NaNO₃, NaCl and MgSO₄, 300 mM fructose, NH₄Cl and KCl, 1 mM sodium saccharin (saccha), 410 mM Na₂SO₄, 3 mM citric acid (H), 10 mM acetic (Acet) and hydrochloric acids, and 1 mM quinine HCl. Mean data are from Frank and Nowlis, 1989.

Figure 4. N Specialist (NaCl-best) and E generalist (HCl-best) hamster chorda tympani nerve fibers transmit distinct information to the hamster brain about ionic taste stimuli
Mean ($\pm$ se) neural response rates to 100 mM sucrose and KCl; 30 mM NaCl, NaNO₃, MgSO₄ and NH₄Cl; 3 mM HCl, acetic (Acet) and citric acid (H), 1 mM quinine HCl; and control spontaneous neural spiking rates for 21 N and 9 E neurons are plotted. Most neurons responding best to ionic taste stimuli responded to NaCl. Data, 3/4th of a sample of 40 taste-responsive CT neurons, are from Frank *et al.*, 1988.

Figure 5. Hamsters distinguish sodium salts from acids and bitter salts better than they distinguish among acids and bitter salts

CTAs to Na⁺ salts generalized specifically to 100 mM NaCl (blue), CTAs to acids and NH₄Cl generalized strongly to 10 mM HCl (red), and CTAs to non-sodium salts generalized strongly to 1 mM quinine HCl (gray). The CS groups learned aversions to 410 mM Na₂SO₄, 100 mM NaNO₃, NaCl and MgSO₄, 3 mM citric acid (H), 10 mM acetic (Acet) and hydrochloric acids, 300 mM NH₄Cl and KCl, and 1 mM quinine HCl. The mean data are from Frank and Nowlis, 1989.

Figure 6. Differences in the tastes of NaCl and NH₄Cl or KCl disappear after treating the tongue with amiloride in rats

An aversion learned to the conditioned stimulus (CS) 500 mM NaCl was specific to NaCl in control rats (Water); but after treating with 100 μ M amiloride (Amiloride), sodium, ammonium and potassium chlorides tasted alike as measured by percent suppression of intake. Values are derived from mean data in Hill *et al.*, 1990.

Figure 7. Blocking ENaC mutes NaCl and LiCl responses of CT N fibers but not CT E fibers, which also detect NaCl and LiCl

After treating the tongue with 100 μ M amiloride (+Amiloride), the remaining amiloride-unsusceptible NaCl response elicits a taste perceptually like non- Na^+ salts (FIG. 6). In effect, amiloride serves as a pharmacological “knockout” of one of several rodent CT ionic taste systems. The specific taste of Na^+/Li^+ salts is eliminated when the tongue is treated with amiloride and N neurons are muted. Mean 5-s response data from Ninomiya *et al.* 1988.

Figure 8. The hamster chorda tympani nerve responds to ionic stimuli that are bitter to people, but does not respond to behaviorally avoided non-ionic bitter stimuli

Quinine and denatonium are avoided behaviorally at 0.3 –1 mM (plum arrow); SOA, *n*-propylthiouracil and caffeine avoided at 1–3 mM (light purple arrow) and cycloheximide avoided at 1 μ M, well below the graph's scale. Mean data ($\pm$ se) are from Frank *et al.*, 2004 and Hettinger *et al.*, 2007.

Figure 9. Stimuli that are bitter to humans form distinct perceptual categories in hamsters
 Ionic bitter stimuli: denatonium, quinine and MgSO₄, and non-ionic sucrose octaacetate (SOA) and caffeine are perceptually distinct to hamsters. Test stimuli were water (control), denatonium and SOA. Quinine and MgSO₄ aversions (CS group) generalized strongly to denatonium but did not generalize to SOA. Caffeine and SOA aversions did not generalize to denatonium, nor did caffeine and SOA aversions cross-generalize. Stimuli were 3 mM denatonium benzoate, 1 mM quinine HCl, 180 mM MgSO₄, 30–100 mM caffeine and 1–1.5 mM SOA. Mean data (±se) are from Frank *et al.* 2004.

Figure 10. Hamster N neurons respond transiently to an anion shift from acetate to chloride with an increase in neural activity after 25-sec adaptation

The reciprocal shift from chloride to acetate did not increase the neural response. Stimuli were 30 mM sodium chloride and sodium acetate ($N = 3$). Mean data are from Rehnberg *et al.* 1993.

Figure 11. Responses of CT Na⁺ specialists to NaCl are stronger in sodium-replete than in sodium-deprived rats

Mean (+ se) response rates for 10 seconds are plotted. The dashed lines indicate mean (+ 2 se) control response levels in sodium-deprived and sodium-replete animals. Test stimuli were 500 mM sucrose, 100 mM NaCl, 10 mM HCl, and 20 mM quinine HCl. Data are from Contreras and Frank, 1979.

S Fibers: 100 mM Sucrose plus Quinine

Figure 12. Responses of hamster sucrose-best specialists to 100 mM sucrose contaminated with increasing levels of bitter quinine
Means $\pm$ se are plotted. The dashed line marks control mean + 2 se, a response level considered to be above background. Data are from Frank, Formaker and Hettinger, 2005.

Table 1
Traits of Rodent Chorda Tympani (CT) Neuron Types.

1	2	3	4	5	6	7	8	9
Type (^H % total)	Sweets	Ionic Stimulus Specificity		Quinine	Thermal	Drug	Dietary	
	HRM _{All}	HR _{Cation}	^H Anion size	HR _{Mix} Sucrose				
Specialist								
S (25)	yes	--	--	inhibits	no	--	R _{Na} deprived	
N (50)	no	Na ⁺ /Li ⁺	no	no	c [†] w [↓]	yes [↓]		yes [↓]
Generalist								
E (25)	no	no	yes	excites	* c [↑]	no		no

Footnotes: 1. explains codes for columns, 2. cites key references.

¹Col. 1, % hamster (H) CT types; col. 2, hamster, rat (R) & mouse (M) CT responses to sweet (like sucrose) stimuli; col. 3, hamster & rat CT-response cation specificity; col. 4, hamster CTresponse/anion-size specificity; col. 5, rat & mouse CT response-threshold for NaCl stimulus; col.6, quinine stimulus inhibits or excites hamster CT response; col.7, rat CT response to temperature (c = cooling, w = warming), increase (†) or decrease (↓); col. 8, ENaC block by amiloride decreases (yes,↓) hamster, rat & mouse CT response; col. 9, sodium deprivation decreases rat CT response.

* Acid-generalist.

²Key references are cols. 1–3: Frank 1973, Frank *et al.* 1988, Lundy & Contreras 1999, Ninomiya *et al.* 1999; col. 4–5: Rehnberg *et al.* 1993, Frank *et al.*, 1983; Yasumatsu *et al.*, 2003; col. 6: Frank *et al.*, 2004; 2005; col. 7: Lundy & Contreras 1999; Breza *et al.*, 2006; 2007; col. 8: Ninomiya & Funakoshi, 1988; Hettinger & Frank, 1990; Yasumatsu *et al.*, 2003; col. 9: Contreras & Frank, 1979.

Table 2

CT Neurons vs. TR Knockout (KO)/Block (BL)

1	M _G PCR KO						Ion Chan. KO/BL			
<i>Taste</i>	<i>Sweet</i>		<i>Bitter</i>		<i>Sour</i>		<i>Na⁺ salt</i>			
<i>Receptor</i>	TIR2-3		TIR1-3		M ₁ PK2		^A ENaC			
Neuron Type	2		3		4		6			
-stimulus	CT	KO	CT	KO	CT	KO	CT	KO	CT	BL
Specialist										
S-sucrose	+	+	+	-						
-glutamate	+	-	+	+						
N-Na ⁺									+	+
Generalist										
E-salts [*]			+	-	+	-	+	-		
-quinine					+	+	+	-		
-acids					+	-	+	+		

CT = chorda tympani; KO = knockout; BL= amiloride block; [CT +] =neural response, [KO (BL) +] = abolish CT response; [+ +, dark fill] =concordance; [+,- light fill] = discordance; M = mice, genetically engineered; A = all 3 rodents: hamster, rat, mouse; MSG +A = Na glutamate + amiloride.

^{*} E-salts include NaCl.