Afferent Gustatory Neural Coding: A Review Article on Dave Smith’s Research

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Spring 2003

A Critical Literature Review submitted in partial fulfillment of the requirements for the Senior Research Thesis
Abstract

The four classical tastants salt, sour, bitter, and sweet are neurally coded through afferent neural processing. The coding of these tastants involves the integration of both across-neuron fiber patterns and labeled-line patterns. The main focus of Dr. Dave Smith’s research involves identifying the neural mechanisms that relate to the detection and discrimination of salts in solutions and compounds. There are at least two transduction mechanisms involved in Na⁺ salt taste quality. In one mechanism, Na⁺ salt is transduced by an apically located amiloride-sensitive ionotropic channel. The other mechanism is classified as an amiloride-insensitive transduction mechanism due to the lack of amiloride in blocking the response to NaCl taste stimuli. Other tastants, such as sweet, sour, and bitter, have their own independent transduction mechanisms. Such mechanisms involve G-protein-coupled metabotropic receptors for both bitter and sweet stimuli and an ionotropic K⁺ channel for acids. Behavioral studies have been conducted on the discrimination between types of salts, such as sodium and non-sodium salts, providing evidence that supports physiological studies on transduction mechanisms and neural coding. This paper will address these types of transduction mechanisms.

This paper will also address the neural coding of the four basic tastants. This research suggests stronger evidence for the across-fiber pattern theory (afferent-neuron pattern theory), but there also appears to be evidence for the integration of both the across-fiber pattern theory (AFP) and the labeled-line pattern theory. That is, certain tastants can be coded by across-fiber patterns and labeled-line patterns, and some tastants
are coded more strongly by the AFP. This paper will also describe the cross adaptation method, which is used to study the characteristics of receptor mechanisms.

This paper will also discuss the nerve responses related to both the AFP and the labeled-line pattern theories. For example, both the hamster NST neurons and the PbN neurons depict best-stimulus categories within the AFP for salt discrimination primarily. Labeled-line pattern theory has been shown to also be involved in nerve responses. This paper will also discuss the effects that neural coding has on how tastants affect behavior. In this paper, behavior includes discriminating between secondary tastes and primary tastes in both salts and other taste stimuli and between inorganic and organic salts. This paper discusses this idea in further detail.

In conclusion, this review will summarize the research of Dave Smith pertaining to afferent neural gustatory coding, with a specific emphasis on the coding of salt.
Introduction

The taste system provides important cues to aid in ingestion of vitamins and mineral nutrients for a healthy body and to avoid toxic chemicals that could harm the body. Taste sensations are associated with specific chemical classes, such as a sweet quality is associated with sugars and carbohydrates. In fact, four basic tastes—sweet, sour, salty, and bitter—define taste quality. Most of these tastants are water soluble and nonvolatile and include anions and/or cations that produce more or less inhibition of neural signaling. All of these tastants are also hydrophilic and consist of weak acids (sour taste), salts (salty and bitter taste), sugars (sweet taste), amino acids (sweet, bitter, umami taste), and proteins (sweet and bitter taste). These tastants dissolve and travel to the microvillar processes of taste receptor cells via saliva and the mucus layer over the taste pore (For more information on regions of taste bud distribution, see “Taste Perception and Taste Bud Distribution” in Smell and Taste in Health and Disease). However, tastants such as quinine (bitter) are lipophilic, or non-water soluble, but they are still just as responsive to salivary flow and the parasympathetic and sympathetic pathways as hypophilic tastants.

As we must be able to discriminate between different tastes, there is not simply one mechanism responsible for the transduction of chemical signals into neural activity. Instead, there are multiple transduction mechanisms designed to recognize specific chemicals. These transduction mechanisms include amiloride-sensitive ion channels, K+ channels, and G-protein-coupled second messenger receptors that will be discussed further in this paper. The output of these transduction mechanisms is the perception of one or more of these tastants, including the detection of both secondary and primary tastes.
Furthermore, the neural conduction of taste signals involves specific nerves. These nerves consist of sensory axons, including the facial, glossopharyngeal, and vagal cranial nerves, and they innervate taste cells. The nerve fibers of these nerves enter the base of the taste bud and form chemical synapses with the basal cells (small, round cells found at the basolateral area of the taste bud). One taste cell may have several synaptic contact sites with afferent nerve fibers. However, the contact only synapses certain fibers (For more information, see chapter 9 of *Smell and Taste in Health and Disease*). That is, the nerves are neuron-specific in their conduction of taste signaling. There are four specific neuron types—N-best (salts), H-best (sours), Q-best (bitters), and S-best (sweets). The output from these neuron types is then sent to the nucleus of the solitary tract (NST) where taste perception occurs. The central processing of these taste types occur in higher processing areas. The NST sends the taste responses to these higher processing areas, particularly the gustatory cortex. The gustatory cortex encodes taste quality and determines the behavioral response to particular ingested tastants.

Moreover, the intensity of a taste quality is also important. For example, a cake without enough sugar is nearly inedible, or meat that is too salty is difficult to ingest. One of the roles of the action potential is to be involved in the quality or intensity of taste stimuli coding. They also help to shape taste stimuli responses, possibly resulting in a rapid excitation upon stimuli onset. The action potential may also be important in transmitter release (See “Taste Perception and Taste Bud Distribution” in *Smell and Taste in Health and Disease*). The anatomy of the gustatory reception system includes the fungiform papillae, which contain the taste buds, which contain the taste receptor cells. Research has been conducted on human fungiform papillae response properties (Bealer
and Smith, 1974) using cross-adaptation techniques that distinguish the responses of both first and second stimuli. Each individual human fungiform papillae mediates more than one of the four classical taste qualities and elicits a high level of responsiveness, which shows that a taste sensation is recognizable in response to more than one taste quality. Correctly identifying a number of stimulus classes can happen only when there is a single taste papilla stimulated with a strong concentration. At weaker concentrations, several papillae are necessary for eliciting taste sensations since these stimuli intensities are below threshold for a single papilla.

This paper reviews research by Dr. Dave Smith on the afferent neural coding of Na+ salts in the gustatory system. Dr. Smith has conducted behavioral experiments in rodent and human models to characterize gustatory perceptual capabilities. In addition, Smith has conducted electrophysiological and neuropharmacological studies of the taste system as a means to better understand the taste pathway organization in the brainstem. The afferent neural coding of all four of the basic taste stimuli, sweet, sour, bitter, and salty, as well as the transduction mechanisms for each tastant will be reviewed. Furthermore, the processing of the afferent neural code in the nucleus of the solitary tract and the parabrachial nucleus will be examined. As Dr. Smith’s research has been the perception and coding of the salt taste quality, the relationship to behavior, including detecting salts among other stimuli and discriminating between salts, and the associated neural code will be specifically examined in this paper.

**Taste Transduction**

This section covers the transduction mechanisms that allow taste perception. There are different transduction mechanisms for four basic tastants, sweet, sour, salty,
and bitter. Transduction involves the processing of the neural signal into an electrical signal, which is sent to the brain for different taste perceptions. These transduction mechanisms are measured using cross adaptation methods. Although this paper explains the research on sour, sweet, and bitter taste transduction mechanisms, Smith’s research is more specifically involved with salt transduction. Thus, this section addresses all taste transduction mechanisms special emphasis on salt transduction.

The cross adaptation procedure is important in the transduction mechanism processing. Cross-adaptation is a psychophysical method used to study the characteristics of these receptor mechanisms. A cross-adaptation procedure involves adapting the lingual taste receptors to one chemical and immediately presenting a second chemical without rinsing. In this procedure a concentrated solution flows over an extended tongue to determine the amount of seconds it takes for the strong intensity of the taste stimuli to fade. That is, the purpose of cross-adaptation is to determine the degree to which adaptation to the first chemical stimulus decreases the response of the second chemical stimulus. Both stimulus and receptor molecules must reach equilibrium, the point at which no additional taste is evoked from continued stimulation with the adapting solution. Then, if the application of a second stimulus fails to evoke a taste assumed that the same receptor mechanism is utilized for detection of the second stimulus. If the second stimulus produces a taste, then it is likely that a new receptor mechanism is utilized for detection that was not involved in the initial response to adaptation. Thus, the purpose of this procedure gives evidence for a common link in the neural mechanisms of the two stimuli (Smith et. al. 1996).
There are figures that depict the transduction mechanisms represented in taste receptor cells (Figures 1 and 2: Kinnamon and Margolskee, 1996). For example, there are several mechanisms that contribute to acid detection in taste receptor cells (Figure 1: Kinnamon and Margolskee, 1996). One mechanism depicts hydrogen ion influx through the amiloride-sensitive Na+ channel, which depolarizes the taste receptor cell producing an action potential (Figure 1: Kinnamon and Margolskee, 1996). Another mechanism for acid detection involves a proton-gated cation conductance (Figure 1: Kinnamon and Margolskee, 1996). Another sour transduction mechanism involves protons blocking K+ channels localized to the apical membrane, which in turn results in depolarization of the taste receptor cell membrane.

Bitter stimuli, such as quinine or divalent salts may also block the apical K+ channels. K+ salts also taste bitter and appear to permeate the apical K+ channels, though it is not certain if these K+ channels are apically located or if some other pathway plays a greater. In fact, the main transduction mechanism for bitter stimuli appears to be metabotropic receptor activation of second messengers such as cAMP and IP3 (Figure 2: Kinnamon and Margolskee, 1996). Denatonium is a substance that stimulates cAMP and IP3 to release intracellular basolateral calcium ion stores, which results in neurotransmitter release (Figure 2: Kinnamon and Margolskee, 1996) and transduction activation. Another bitter transduction mechanism includes bitter receptor coupling to gustducin and/or transducin to activate the substance PDE, which decreases TRC cNMP levels (another metabotropic receptor protein coupled to gustducin). If cNMPs are inhibited, phosphorylation of TRC receptors and channels are diminished, as well. Such
decreased cNMPs could result in depolarization or hyperpolarization, which results in an action potential and the transduction process.

Sweet stimuli also appear to be mediated by second messengers such as cAMP and IP3, as well as by an amiloride-blockable, second-messenger-independent sweet transduction mechanism (Figure 2: Kinnamon and Margolskee, 1996). This amiloride-blockable transduction mechanism is activated by sugar and sweetener stimuli binding to the taste receptor cell, and providing a buildup of protons that leads to depolarization of the taste receptor cell membrane. In another mechanism, only sucrose binds to the taste receptor cell, stimulating IP3 and a protein kinase enzyme, whose actions produce depolarization of the membrane (Figure 2: Kinnamon and Margolskee, 1996). In a third mechanism for sweet transduction, sweetener stimuli bind to the taste receptor cell, stimulating IP3 to release calcium ion stores, which results in neurotransmitter release (Figure 2: Kinnamon and Margolskee, 1996).

Salts appear to be blocked by amiloride (Figure 1: Kinnamon and Margolskee, 1996). That is, salts appear to have at least two transduction mechanisms. One mechanism for Na+ salt transduction involves an amiloride-sensitive ion channel on the apical receptor cell membrane (Avenet and Lindemann 1988). Treating the tongue with amiloride has no effect on the responses to non-Na+ stimuli but almost completely blocks the responses to organic Na+ salts such as Na-acetate or Na-gluconate (Formaker and Hill 1988). Further evidence shows that amiloride decreases the total NaCl and Na-gluconate intensities (Ossebaard and Smith 1995). The suppression of the total Na-gluconate intensity is greater than that of NaCl across all concentrations, which suggests that in humans the activation of amiloride-sensitive channels plays a greater role in Na-
gluconate than in NaCl transduction (Ossebaard and Smith 1995). This data further implies that apical amiloride-sensitive channels play a role in NaCl and Na-gluconate transduction in humans. The activation of amiloride-insensitive pathways must play a role as well because the suppression of the perceived intensity of NaCl and Na-gluconate is not complete (Ossebaard and Smith 1995a).

Further evidence exists for transduction pathways other than amiloride-sensitive ion channels in determining the saltiness of salts such as Na+ and Li+ salts (Ossebaard and Smith 1996). One study has shown that the total taste intensity of NaCl and LiCl is reduced by amiloride, yet amiloride does not affect KCl. In fact, in another study, amiloride treatment decreased the total NaCl and Na-gluconate intensities, but had no effect on the total KCl intensity, which suggests that humans do not have an amiloride-sensitive transduction component for KCl (Ossebaard and Smith 1995a). Also, the suppression of the total Na-gluconate intensity was greater than that of NaCl across all concentrations, which suggests that in humans the activation of amiloride-sensitive channels plays a greater role in Na-gluconate than in NaCl transduction. This data further implies that apical amiloride-sensitive channels play a role in NaCl and Na-gluconate transduction in humans. But, the activation of amiloride-insensitive pathways must play a role, as well, because the suppression of the perceived intensity of NaCl and Na-gluconate is not complete. The larger amiloride insensitive channel for NaCl and Na-gluconate may stem from differences in Na+ salt transduction between rats and humans (Ossebaard and Smith 1995a).

In addition, there is a hydrogen ion transduction mechanism for coding sour taste quality. However, this transduction of hydrogen ions is not the only mechanism that can
result in the sour taste quality (Ossebaard and Smith 1996). The sournes of Na+ and Li+ salts is also mediated by amiloride-sensitive channels on the apical membrane of taste receptor cells. The saltiness quality of these salts may come from other mechanisms, however, because it does not appear to depend on amiloride-sensitive transduction components. There is no evidence for an amiloride-sensitive transduction component for acids in humans, however (Ossebaard and Smith 1995a, 1996).

In fact, in some mammalian species, amiloride reduces the NaCl response by 60% (DeSimone et al 1984, 1993), which is thought to arise via a paracellular transduction pathway that is insensitive to amiloride (Elliott and Simon 1990). In this paracellular pathway, the larger organic salts are restricted to this vicinity because the large anions that comprise these organic salts cannot penetrate the tight junctions between the receptor cells. Thus, the amount of Na+ flow through this pathway is limited. In other words, there are at least two mechanisms for Na+ salt transduction in which small anions stimulate via the submucosal amiloride-insensitive pathway and larger anions, such as Na-gluconate, stimulate via the apical amiloride-sensitive ion channel. So, these differences in amiloride sensitivity may reflect differences in the contributions of these and other Na+ transduction mechanisms. The three salts—NaCl, Na-gluconate, and KCl—showed a salty taste after distilled water without amiloride treatment. These salts also showed a sweet, bitter, or sour side taste after the distilled water was given. Although the sweet and bitter side tastes were unaffected, amiloride treatment greatly reduced the sour side taste of NaCl and Na-gluconate salts. KCl also showed a great sour side taste, as well as citric acid, but both were unaffected by amiloride treatment. Therefore, amiloride has a specific effect on the sour side taste of sodium salts, which
suggests that the sourness of these salts may exist with the activation of amiloride-sensitive transduction mechanisms. KCl sourness appears to be regulated by pathways insensitive to amiloride. Thus, amiloride has no effect on the saltiness of NaCl in humans when humans are asked to assign tastes intensities of NaCl, but instead amiloride has a specific effect only on the sour taste of salt.

Although there is a hydrogen ion transduction mechanism for coding sour taste quality, this transduction of hydrogen ions is not the only mechanism that can result in the sour taste quality (Ossebaard et. al. 1997). The sourness of Na+ and Li+ salts is also mediated by amiloride-sensitive channels on the apical membrane of taste receptor cells in hamsters. Recall that amiloride blocks the acid responses in hamsters. In fact, research has found that amiloride blocks sodium channels in some taste receptor membranes, such as in the hamster. However, humans lack the sodium-specific neuron type that is evident in the hamster. Mechanisms for human saltiness continue to be studied, especially in humans. Thus, the characteristics that mediate human perception of saltiness are not known with certainty.

The saltiness quality of salts may come from mechanisms other than amiloride-sensitive channels; saltiness does not appear to depend on these transduction mechanisms. There is no evidence for an amiloride-sensitive transduction component for acids in humans (Ossebaard and Smith, 1995a, 1996).

One study examines the effects of amiloride treatment on the responses of central taste neurons to acids and NaCl, and it demonstrates how input from amiloride-sensitive transduction mechanisms is organized across the various gustatory neuron types in the medulla (Boughter and Smith 1998). The responses of NaCl and the two acids HCl and
citric acid are reduced by amiloride, which suggests two transduction mechanisms for acids in the hamster (Boughter and Smith 1998). But, amiloride did not affect the responses to any of these stimuli in the H-best neurons or in the Q-best neurons, which suggests that the receptor mechanisms that provide input to these neuron types are segregated from the amiloride-sensitive apical ion channel. Thus, the H- and Q-best neurons receive input from taste receptors lacking amiloride-sensitive mechanisms, and they transduce Na+ and H+ responses by paracellular channels or by unidentified apical cation channels, whereas taste receptor cells with amiloride-sensitive transduction mechanisms provide input to N-best cells of the taste NST (Boughter and Smith 1998).

However in this study, S-best neurons are shown to be unsuppressed by amiloride in acids, as happened with NaCl in S-best cells, which suggests convergence of the CT fibers onto NST neurons. The majority of the information from the responses of S-best NST neurons to acid must arise from input from H-best CT fibers, and the amiloride-sensitive NaCl responses of the S-best NST cells must arrive mainly from the N-best CT fibers. All of this data shows great organization of taste sensitivity in NST neurons and reflects great specificity. In other words, input from the amiloride-sensitive channel is specifically funneled into two NST cell types and is completely restricted from the other two types. However, discrimination among different tasting stimuli depends on the relative activity in different neuron types because differential activity must be generated across the afferent neurons (Smith 1985; Smith and Frank 1993). For example, when NaCl response is blocked by amiloride in N-best neurons, the remaining neurons cannot provide for the NaCl/KCl discrimination because these salts are not differentiated by these neurons.
In addition, there appear to be two amiloride responses in the rat NST. This segregation consists of amiloride-sensitive and insensitive responses in the rat NST (St. John SJ and Smith DV 2000). One study examined the relative contribution of two transduction pathways—amiloride-sensitive and amiloride-insensitive—to salt discrimination NaCl responses in the rat NST. This study found that the pathways are partially inhibited by amiloride. The results showed that responses in N-best neurons were predominantly affected by amiloride. However, this study did not show the absolute segregation of amiloride-sensitive and insensitive pathways. The average responses to NaCl were reduced by amiloride in all neuron types, which suggests some convergence of amiloride-sensitive and insensitive peripheral fibers onto H-and S-best NST neurons. Most of the amiloride-sensitive input goes to N- and S-best neurons, predominantly to N-best neurons, and the input to H-best neurons is very low (St. John SJ, Smith DV 2000).

Finally, recall that stimuli can include anions and/or cations. These anions and cations are important in inhibiting certain tastants. As the anion size is increased, the cation’s taste is reduced because the larger anions produce more inhibition. For example, NaCl is saltier than sodium acetate because the chloride anion (Cl-) is smaller than the acetate anion. Thus, the acetate anion produces less inhibition that the sodium cation produces. Furthermore, as anions get larger, not only do they inhibit cations, they also take on bitter and sweet tastes, depending upon their structure. As cations get larger, they not only retain a salty taste, but they also take on a bitter taste. For example, lithium is a cation smaller than sodium, but it is also toxic. Therefore, anions and cations, such as
chloride, sodium, and potassium ions are important in transducing neural signals to electrical signals for taste perception.

**Neural Coding of the Gustatory System**

Afferent neural activity in the brain plays a key role in coding taste information regardless of the stimuli transduction or how the stimuli is processed. The main question that dominates gustatory physiology is in determining how tastants are coded. Research has examined the role of both across-fiber patterns and labeled-line patterns in the coding of gustatory signals. Studies have shown that the across-neuron pattern theory and the labeled-line theory are both responsible for taste quality coding (Smith et. al 1983a). However, for salt discrimination, the across-neuron pattern theory seems more relevant. Recall that the across-neuron patterns seem to arise from the responses of particular classes of neurons and appear to be dominated by them (Smith et. al 1983a). In the responses of hamster NST neurons, the best-stimulus categories of neurons within the across-neuron pattern have been identified and have been shown to elicit a best-stimulus pattern from that best-stimulus category (Travers and Smith 1979).

In addition, neurophysiological studies have shown that gustatory neurons respond best to certain tastants, which provides support for the labeled-line pattern of coding (Smith et al 1983a). Labeled-line activity involves neural activity that represents taste in only one class of stimuli such that those gustatory neurons fit into a best stimulus category based on the maximum response that they elicit. The main criticism against labeled-line theory includes dividing neuron populations into a best stimulus criteria. For instance, a sweet tastant can be coded by activity in sucrose-best fibers, salt tastants can
be coded by activity in NaCl best fibers, etc. (Smith et al 1983a). Researchers of the labeled-line theory have demonstrated more interest in the effects of only four tastants (sweet, sour, bitter, salty) rather than how a response can vary within the tastant (Smith et al 1983a). There has been no evidence for labeled-line sufficiently existing without across-fiber pattern.

Smith et al. conducted a study showing similarities in the neuronal response profiles in the NTS and PbN of the hamster using multivariate statistical techniques (Smith 1985). They found that best-stimulus categories exist in the NTS and PbN cells and that this categorization also occurs together with cluster membership in 80% of the cases. Thus, their research supports the existence of neuron types in the gustatory system in hamsters. Their results showed that taste neurons in the hamster brainstem can be grouped into categories based on their response profiles (Smith 1985). The hamster contains sucrose-sensitive compartments unlike the NaCl- and HCl-sensitive neurons, which show more similarity to one another than to sucrose.

In addition, sucrose and QHCl are suppressive in their effects on taste-responsive cells in the PbN when presented as a mixture. QHCl can suppress sucrose, but the activation used by QHCl to suppress sucrose is external to the sucrose-responsive neuron, which suggests that it arises from inhibitory influences from other cells (Smith et. al 1994).

Further studies also show that there are multiple gustatory sensitivities. For example, one study shows that taste receptor cells are responsive to stimuli representing more than one of the four classical taste qualities. In addition to saltiness, salts have significant sweetness, sourness, or bitterness (Smith and McBurney 1969). Thus, a
specific taste quality cannot be uniquely related to the transduction of a molecule. On the other hand, a certain taste sensation could arise via several different transduction mechanisms (Smith and van der Klaauw 1995). For example, KCl, which increases following NaCl adaptation, is unlikely to arise via the same transduction mechanism as the bitter taste of quinine. Thus, taste must emerge from neural processing that is beyond the receptor (Smith and van der Klaauw 1995). This information strongly suggest that stimuli representing the human qualities of sweet, salty, sour, and bitter and those taste qualities are not restricted to specifically tuned cell types (Gilbertson et al 2001). In addition, this broad responsiveness may stem from transduction mechanisms that overlap for the different classes of stimuli within a single receptor cell (Gilbertson et al, 2001). For example, cells that exhibit voltage-activated Na+ currents are shown to be more broadly tuned than those with only K+ currents, which could be from the more broadly tuned cells being more mature than those without Na+ currents. Therefore, this study clearly shows that these cells are not specific to a single stimulus (Gilbertson et al, 2001).

Moreover, studies have also been conducted on other nerve responses. Studies have been conducted on hamster chorda tympani nerve fibers, which are highly varied in ability to respond to taste stimuli that are sweet, salty, sour, or bitter to humans (Frank et al. 1988). Yet, with higher dosages of amiloride, there is suppression of the chorda tympani nerve responses (Smith et al. 1996). These variable responses could stem from the side tastes elicited in tastants. For example, many salts have a salty sensation, even those with other cations, like KCl and NH4Cl, but they also elicit a large bitter component as well (Smith and McBurney 1969). However, not all Na+ salts have only a salty component, such as NaNO3 and Na2SO4, which have large sour or bitter qualities
Halide salts have a bitter component related to increasing cationic and anionic weight, and saltiness is reduced greatly with heavier anions (Murphy et. al. 1981). Many organic salts, such as Na-gluconate and Na-acetate, are salty and sour (Ossebaard and Smith 1995b). Therefore, salts produce complex tastes, such as sweet, sour, bitter, in addition to salty.

Furthermore, mammalian taste neurons respond to stimuli representing more than one of the classical four tastants—sweet, sour, bitter, and salty (Smith and Travers 1979, Smith et al. 1983a, Travers and Smith, 1979, Van Buskirk and Smith, 1981). According to the labeled-line hypothesis, a taste quality is represented by activity in one of the best-stimulus fiber groups (Smith et al. 1983a). For instance, in studies of the hamster NST and PbN (Smith and Van Buskirk 1981), response profile analysis of taste responsive neurons suggest that these cells are grouped into “types” at each level of response profiles. That is, the hamster contains sucrose-sensitive compartments unlike the NaCl- and HCl-sensitive neurons, which show more similarity to one another than to sucrose (Smith 1985).

Another coding of neuron types is the across-neuron pattern. The across-fiber patterns seem to arise from the responses of particular classes of neurons and are dominated by them (Smith et al. 1983b). In the responses of hamster NST neurons, the best-stimulus categories of neurons within the across-neuron pattern were identified and elicited by a best-stimulus pattern from that best-stimulus category (Travers and Smith 1979). This study dealt solely with the brain stem responses of the four basic taste stimuli. Later, another study was done and found that hamster PbN neurons that are best for a certain stimulus are among the most responsive in its across-fiber patter (Van
Burskirk and Smith 1981). If these best neurons were not present, then the across-neuron patterns evoked by that class of compounds would be more dissimilar than the patterns evoked across the entire population sample of neurons. Thus, this suggests that neurons identified by their best stimulus were critical to the establishment of the across-neuron pattern. For example, sweet compounds would produce only highly similar across-neuron patterns when the “sucrose-best” neuron class is present in order to help define the similarities in the patterns (Van Burskirk and Smith 1981).

This study showed that certain neuron groups dominate the across-neuron patterns elicited by certain stimuli (Smith et al. 1983b). For instance, sweet-tasting compounds elicited a certain pattern in which the neurons were most active and those compounds belonged to the S-neuron group, which resulted in a tight grouping of these stimuli in the stimulus space. Without the S-neuron group, the across-neuron pattern evoked by sweet-tasting compounds disperse within a multidimensional stimulus space. That is, the S-neuron class is a necessity in establishing similar across-neuron patterns among the sweet-tasting stimuli.

Furthermore, the across-fiber pattern that nonsodium salts and acids elicit are dominated by the H-neurons (Smith et al. 1983b). Without the H-neuron group, the across-neuron patterns evoked by the acids disperse within a multidimensional stimulus space. Thus, the H-neuron class must also be a necessity for establishing similar across-neuron patterns among the acids and nonsodium salts. The N-neurons dominated the across-neuron patterns evoked by the sodium salts, but it was not as striking as with the S- and H-neuron classes.
In addition, this study (Smith et al. 1983a) also addressed the coding of taste quality in the labeled line pattern theory. This study shows that labeled lines are specifically tuned to one class of stimuli. However, studies have shown that mammalian taste afferents usually respond to multiple classes of stimuli, which are modulated by stimulus concentrations (Travers and Smith 1979 and Van Buskirk and Smith 1981). Thus, these researchers argue for across-neuron patterns because these neurons are too unclear in their responsiveness to serve as labeled-lines for taste quality. The results of this study suggest that both labeled-line and across-neuron patterns of activity occur (Smith et al. 1983b). That is, similar-tasting compounds evoke highly similar across-neuron patterns in the PbN, but also these patterns are dominated by activity in certain neuron classes. Although the two pattern theories have different perspectives on the roles for taste neurons, this study suggests that taste discrimination depends on comparing activity in different neuron classes, which is compatible with either pattern theory.

Nerve Responses for Tastants

Moreover, according to intracellular recordings of numerous species, taste cells show that most taste stimuli elicit slow, depolarizing receptor potentials and associate with increases or decreases in membrane resistance. A single taste cell responds to a variety of taste stimuli representing more than one of the four basic taste qualities, and the receptor potential shows slow adaptation despite continuous stimulation. Primary afferent nerve recordings decline with repeated application of taste stimuli. Under the
right conditions, receptor potentials at the apical membrane of the taste receptor cell\textsuperscript{1} can elicit action potentials, making the membrane responsive to taste stimulation. At the basolateral membrane, the taste receptor potential located on the apical membrane must be conducted to sites of transmitter release. In fact, taste cells in both mammals and amphibians are electrically excitable and contain voltage-dependent Na\textsuperscript{+}, K\textsuperscript{+}, and Ca\textsuperscript{2+} currents. These voltage-dependent conductances ensure that the action potential will be elicited by the receptor potential at the apical membrane.

Furthermore, one specific nerve involved in nerve responses for perceiving tastes is the superior laryngeal nerve (SLN) fiber. The SLN responses are closely related to stimulus concentration, but they are not easily classified according to their response profiles; thus, they are not easily grouped into neuron types. That is, these nerves are not do not respond to simply one tastant. For example, if a salt is ingested, the salt may also detect other tastants, or secondary tastes, such as sour or bitter, which prevents just one taste perception. In addition, the chemical sensitivities of the SLN appear to be quite different. For example, other studies have shown that these fibers do not discriminate among taste qualities in the same way as other taste nerve fibers because they are not separated into classes based on their response profiles (Dickman and Smith 1988). However, more recent classification of SLN fibers classifies these fibers according to which stimuli they are the most responsive (Smith and Hanoamori 1991). Yet, the problem is that the classification is not very discrete. The most variances can be accounted for by three factors—water, acid, and salt sensitivities. However, any one

\textsuperscript{1} A taste bud cell is comprised of two sections—an apical region and a basolateral region. The apical region contains the cell membrane of the receptor, and the basolateral region contains the second messenger systems and the inside of the receptor membrane.
fiber may be derived from any two of these processes. Thus, some cells are responsive exclusively to water, salt, or acid, and other cells share two or more of these sensitivities (Smith and Hanoamori 1991).

In addition, the across-fiber patterns seem to arise from the responses of particular classes of neurons and appear to be dominated by them (Smith et al. 1983b). In the responses of hamster NST neurons, the best-stimulus categories of neurons within the across-neuron pattern were identified and elicited by a best-stimulus pattern from that best-stimulus category (Travers and Smith 1979). Also, another study was done and found that hamster PbN neurons that are best for a certain stimulus are among the most responsive in its across-fiber pattern (Van Burskirk and Smith 1981). If these best neurons were not present, then the across-neuron patterns evoked by that class of compounds would be more dissimilar than the patterns evoked across the entire population sample of neurons. Thus, this suggests that neurons identified by their best stimulus were critical to the establishment of the across-neuron pattern (Smith et al. 1983b). Thus, certain neuron groups dominate the across-neuron patterns elicited by certain stimuli.

However, in a study done on transduction mechanisms in the NST of rat brain stems, there was no support for the labeled line in the NST (St. John and Smith 2000). Information about NaCl is carried mainly through N-best cells, but this is not true for KCl because KCl evokes equivalent levels of activity in N- and H-best cells and even more activity in N- and S-best cells combined than in H-best cells alone. Also, amiloride does not just reduce N-best neural activity to zero while having no effect on H- or S-best neurons, but rather it reduces both N-best and S-best responses without eliminating activity in either neuron type completely. Therefore, this study strongly suggests that it is
not the absolute activity in any single neuron type, but it is the relative activity across neurons that represents the taste of these salts (St. John and Smith 2000).

Moreover, the activity in any once cell is usually unclear with respect to taste quality because different stimuli can produce the same impulse frequency in a neuron given at appropriately chosen concentrations (Travers and Smith 1979, Van Buskirk and Smith 1981). Also, this study (Smith et al. 1983b) showed that the across-neuron pattern created in any single cell class can be unclear in taste quality. For example, across the S-neuron group, sucrose and citric acid produced similar activity. The across the H-neurons, the sodium and nonsodium salts and acids also evoked highly similar patterns of neural activity. Therefore, more than one neuron type is necessary for the pattern in order to distinguish the patterns evoked by dissimilar stimuli (Smith et al. 1983b).

Furthermore, studies reveal that that a best-stimulus category corresponds with each of the four taste stimuli (sucrose, NaCl, HC1Cl, and QHCl) (Smith et al 1983b). In one study, the best-stimulus categorization and cluster membership agree in 80% of the cases at both levels. In other words, the results of the cluster analysis reveal that there appears to be three clusters of neural response profiles at both the NST and PbN and that they are characterized at both synaptic levels by their main sensitivity to sucrose and other sweet-tasting compounds, sodium salts, and nonsodium salts and acids. However, this correspondence does not support the existence of neuron groups. What it does support is a trend from peripheral to medullary to pontine levels in hamster taste neurons, whether by best stimulus or by cluster membership (Smith et al. 1983b).
Relationship to Behavior: Detecting Salts and Discriminating Between Salts

Other studies use magnitude estimates to show that the number of taste response categories is responsible for the differences in the amiloride effects (Ossebaard et al 1997). For example, in one study, amiloride suppressed the perceived saltiness only if saltiness was rated according to the magnitude estimate scale. Amiloride suppressed sourness if all taste qualities were rated, or if sourness alone was rated (Ossebaard et al 1997). The magnitude estimates of the sourness of LiCl by the sour-only group were greater than those by the profile group, which suggests that the subjects can also combine sensations when asked to rate only LiCl sourness (Ossebaard et al 1997).

In addition, salts can produce side tastes. The three salts—NaCl, Na-gluconate, and KCl—show a salty taste after distilled water is presented without amiloride treatment (Ossebaard, Smith 1994). These salts also show a sweet, bitter, or sour side taste after distilled water is given. One study showed that although the sweet and bitter side tastes were unaffected, amiloride treatment greatly reduced the sour side taste of NaCl and Na-gluconate salts (Ossebaard, Smith 1994). In this study, KCl also showed a great sour side taste, as well as citric acid, but both were unaffected by amiloride treatment. Therefore, amiloride has a specific effect on the sour side taste of sodium salts, which suggests that the sourness of these salts may exist with the activation of amiloride-sensitive membranes. KCl sourness appears to be regulated by pathways insensitive to amiloride. Thus, amiloride has no effect on the saltiness of NaCl in humans when humans are asked to assign tastes intensities of NaCl, but instead amiloride has a specific effect only on its sour taste. Further, there should be no confusion in the perceived taste qualities of sourness and saltiness because amiloride affects both saltiness and sourness, and the
effect of amiloride is specific to the sourness of NaCl and Na-gluconate (Ossebaard, Smith 1994). Moreover, amiloride strongly suppresses the sour portion of NaCl, and the sourness of LiCl is almost completely blocked by amiloride; the sourness of LiCl appears to be greater after water adaptation than that of NaCl. The sourness of LiCl and NaCl seems to come from a different transduction mechanism than the sourness of citric acid, which was not affected by amiloride treatment. This lack of a sourness effect is supported also by psychophysical data from Ossebaard and Smith’s study (1995a). Although there appears to be different transduction mechanisms for citric acid and NaCl/LiCl sourness, the sensations may still be the same such that humans can distinguish citric acid sourness from NaCl/LiCl sourness. There was no effect on the saltiness of NaCl or LiCl either, except for a small decrease in saltiness in the 0.1M NaCl, which was used as an example and modulus for saltiness. This small reduction in the saltiness of 0.1M NaCl provides little support for amiloride suppressing the saltiness of NaCl. The saltiness of all LiCl concentrations were unaffected by amiloride. However, the sourness of LiCl, as well as NaCl was greatly affected by amiloride treatment. In fact, the amiloride-sensitive epithelial ion channel is permeated more by Li+ than Na+. Thus, this data combined with the present study data reveal that the amiloride-sensitive epithelial ion channel is a main mechanism in perceiving sourness in NaCl and LiCl and has only a small role in perceiving the saltiness of NaCl and LiCl. Also, blocking the apical ion channels results in a strong suppression of the sour taste in sodium salts, but it has no effect on the salty quality, supporting the existence of an amiloride-sensitive transduction component in humans.
Furthermore, amiloride and a stimuli mixture for both NaCl and KCl show a dose-dependent decrease in the response of the N-best neurons (Smith and Boughter 2000). In addition, there are small effects on the S-best neurons, a large decrease in N-best cells, and a somewhat decrease in amiloride when amiloride is mixed with a stimulus. Thus, N-best activity is necessary to establish distinct across-neuron patterns between NaCl and KCl. However, these considerations do not prove that N-best activity alone is enough for the distinction between NaCl and KCl. That is, the response of any one neuronal type is ambiguous regarding both the intensity and the quality of the stimulus (Smith and Boughter 2000).

In one study conducted in the rat NST, amiloride had a concentration-dependent effect on the neural representation of NaCl and KCl such that at higher concentrations, behavior, implying neural mechanisms that allow salt detection, seemed to improve (St. John and Smith 2000). In fact, there are two stages involved in salt detection. In the first stage, the neural element is affected by NaCl adaptation. This neural element plays necessary part in perceiving saltiness because NaCl adaptation eliminates the saltiness of all stimuli. In the second stage, adapting the taste cell reduces the excitability of the entire receptor cell (Smith and van der Klaauw 1995a). Therefore, these ideas conclude that the saltiness of any stimulus is determined by the degree to which it activates NaCl-responsive taste receptor cells (Smith and van der Klaauw 1995a). For example, adapting inorganic salts, such as Na+, K+, NH4+, and Ca2+ salts, to NaCl eliminates their perceived saltiness (Smith and McBurney 1969), and the other taste qualities that these salts elicit are either unaffected by NaCl adaptation or increased because of NaCl adaptation.
Further, the purpose of one study was to examine the relationship between the saltiness of NaCl and the saltiness of the more complex organic salts, including acetate, citrate, tartrate, ascorbate, and glutamate anions, and to determine if NaCl adaptation reduces the saltiness of these stimuli as shown for inorganic salts (Smith and McBurney 1969). This study found that NaCl adaptation reduced the saltiness of all salts, despite their specific cation or anion. Salts including KCl, NH4Cl, NaTar, NaCit, or MSG did not taste salty after the tongue was NaCl adapted. NaCl and LiCl had the greatest reduction in total intensity estimates after NaCl adaptation (Smith and van der Klaauw 1995a). Thus, NaCl adaptation prevented the perception of a salty taste quality. Another study also showed that the Na+ salt responses were reduced by NaCl adaptation in all cells that responded to NaCl, no matter what their best-stimulus classification (Smith et al. 1996). Another study examines salt discrimination using direct magnitude estimation for the taste quality profiles for 15 organic and inorganic salts (Smith and van der Klaauw 1995b). This study found that most salts elicited taste qualities other than saltiness even when NaCl was expressed as sour at most concentrations. Not all salts were mainly salty and taste qualities changed depending on the concentration given. For example, NaCl is a little sweet at low concentrations and becomes really salty at higher concentrations, as well as having a sour side taste. NH4Cl and KCl were salty and bitter and LiCl was salty and sour at higher concentrations. Na+ and Li+ salts had complex taste profiles, with either saltiness or sourness as the main quality at most concentrations. The two Ca2+ salts were bitter over most of their concentrations. All of the Cl-(a simple halide salt) salts except CaCl2 were saltier than their nonhalide counterparts, and the organic salts were a lot less salty than the inorganic salts. Therefore, this study shows that the organic
Na+ and Li+ salts are a lot less salty and more sour than NaCl. Na-gluconate, on the other hand, was both salty and sour, and the perceived sourness of Na-gluconate was blocked by amiloride, as was NaCl, but their saltiness was not affected (Smith and van der Klaauw 1995b). Perhaps further research can investigate the neuroanatomy of salt processing to determine if the transduction mechanisms are so similar for salt and sour that they overlap, with salt being intensified more because of its chemical makeup.

Finally, there is more involved in distinguishing between different tastants, specifically salts, than one transduction mechanism or one neuron type. This idea can be further addressed with research that identifies certain tastants mimicking each other. For example, in one study, amiloride made NaCl taste like KCl in the rat model. For instance, when the researchers modeled the effect of blocking amiloride-insensitive transduction pathways, the neural distinction between NaCl and KCl decreased (St. John and Smith 2000). Thus, NaCl and KCl tasted the same. Therefore, this research further suggests that amiloride has an effect on the ability to discriminate between tastants. This research also implies that the behavioral responses to these two different salts are similar, as well. In addition, this study also suggests that NaCl and KCl have not only similar transduction mechanisms, but also distinct neural coding that allows these salts to be discriminated even without the presence of amiloride. Therefore, further research could investigate the chemical makeup of both salts and how one mimics the other. Also, further research could be done on other tastants to determine if certain sours, for example, mimic each other.
Conclusions

Smith’s research helps to explain the neural mechanisms underlying the afferent gustatory neural coding, with particular interest in salts. The transduction of stimuli begins the taste processing mechanisms. As the neural input from taste stimuli ascends into the gustatory pathway, the taste receptor cell’s responsiveness becomes more complex. The central neurons are even more broadly tuned than the peripheral taste fibers, and the central neuronal responses are modified by changes in stimulus concentration. The intensity of the stimuli is understood in the central neurons, as well. The gustatory system does not rely merely on individual neurons to code information, but it integrates both the labeled-line pattern theory and the across-neuron pattern theory. That is, some taste stimuli not only have their main taste quality, but also elicit secondary tastes. For example, most salts elicit taste qualities other than saltiness, and this salty taste quality changes depending upon the concentration. As concentration increases, the secondary tastes are detected more, as well (For further information, see Smith DV, St. John SJ 1999, Review). Perhaps further investigation of the neural coding of these secondary tastes can lead to more descriptive features as to why the neural processes of tastants can detect more than one tastant at different intensities simultaneously.

In addition, there are more than one transduction mechanism for the neural processing of the four basic tastants. Acids have at least two mechanisms of transduction, but more research needs to be conducted on the cAMPs involvement in transduction. Also, sugars have three transduction mechanisms, and salts have at least one transduction mechanism, as well as sour stimuli.
The ability to discriminate between salts and salt’s affects on behavior also need further investigation. Perhaps the ability for not discriminating between salts involves deficiencies in one or more of the gustatory nerves themselves, and perhaps these deficiencies result in more or less salty preferences. Furthermore, as suggested earlier in this paper, further research should be conducted on the secondary coding of tastes in comparison to the primary tastes to determine if the neuroanatomy overlaps. If an overlap exists, perhaps it results in depiction of more than one taste. On the other hand, such an overlap could be a result of a deficiency in the gustatory nerves.

In conclusion, there appear to be more than one way of transducing a tastant to a neural signal that is then sent to the brain for different taste perceptions. There are also differences or even combinations in both the across fiber pattern and labeled-line pattern theories in coding these responses. The nerves involved in perceiving taste vary in their responses, such as with the best-stimulus categories in the NST and the PbN of hamsters and rats. In Smith’s research, afferent-neuron pattern theory (across fiber pattern theory) plays the main role in the neural coding of tastants. The gustatory afferent neurons and the transduction mechanisms that send the electrical signals to the nerves in the brain also involve the afferent-neuron pattern theory. The behavioral responses that result from transduction and neural coding help to not only discriminate between different tastants, but also help to discriminate between similar tastants. In addition, humans as well as other species have shown that amiloride treatment disrupts the ability to discriminate between sodium and non-sodium salts (For further information, see Smith DV, St. John SJ 1999, Review), which implies that there are different transduction mechanisms for
sodium and non-sodium salts in nerve fiber responsiveness, and, thus, differences in neural coding and the behavioral responses of different tastants.

References


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