THE PSYCHOPHYSICS OF SALT TASTE TRANSDUCTION PATHWAYS

By

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THE PSYCHOPHYSICS OF SALT TASTE TRANSDUCTION PATHWAYS

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Salt stimuli can activate two transduction mechanisms in the rat's oral cavity. One mechanism appears to rely on taste receptor cells that contain sodium-selective ion channels on their surface. Passage through these channels can be blocked with the drug amiloride. The other mechanism is thought to be less selective, with associated fibers responding to potassium and ammonium as well as sodium salts. Most data suggest that the salt responsiveness of this population of fibers is not significantly attenuated with amiloride treatment, although some researchers have found evidence to the contrary. The two salt transduction pathways are commonly grouped according to their amiloride sensitivity as either amiloride-sensitive (AS) or amiloride-insensitive (AI). Activation of the AI pathway appears to be limited by anion size, with large anions like gluconate producing the greatest suppression. Previous research has indicated that the AS pathway is necessary and sufficient for normal sodium detection in the rat as well as necessary for sodium recognition. The current experiments were designed to determine whether the AS

pathway was also sufficient for sodium recognition as well as to elucidate possible functional roles of the AI pathway regarding the perception of nonsodium salts. This was accomplished by observing the effects of physiological manipulations like gustatory nerve transection, amiloride treatment, and acute sodium depletion on the taste-guided behavior of highly-trained rats. Briefly, the sodium-specific AS pathway appears to be sufficient for sodium recognition in acutely depleted animals. In addition, AI receptor cells innervated by the chorda tympani (CT) nerve were found to be necessary for normal detection of ammonium chloride (NH_4Cl) and AI cells innervated by the facial nerve were both necessary and sufficient to discriminate NH_4Cl from KCl. This finding suggests that taste receptor cells innervated by the facial nerve could use separate AI transduction mechanisms with different selectivities for ammonium and potassium. This work also supports the hypothesis that amiloride does not significantly impair the perception of nonsodium salts, as well as the contention that the facial nerve may provide unique information about taste quality in spite of innervating only about 30% of the taste buds in the oral cavity.

CHAPTER 1
LITERATURE REVIEW

Introduction

The current experiments were designed to relate events at the level of the taste receptor cell due to salt stimulation with the perception experienced by the animal, in this case the Sprague-Dawley rat. To this end, performance was measured on a variety of psychophysical tasks including detection threshold, discrimination and recognition on a brief-access test using several salt stimuli. The effects of physiological manipulations such as gustatory nerve transection, oral application of the ion channel blocker amiloride, and acute sodium depletion on taste-guided behavior were also assessed. Prior to elaborating on the details of these experiments, the remainder of this chapter consists of a brief introduction to the anatomy and physiology of the mammalian gustatory system as well as taste coding theory for readers that might be unfamiliar with these concepts.

Peripheral Gustatory System

Taste Buds

In mammals, the sensory cells of the gustatory system are found in clusters of approximately 50 epithelial cells called taste buds (Miller, 1995). Some of these modified epithelial cells are taste receptor cells (TRCs) and contain receptors on their apical membranes capable of interacting with taste compounds. Taste stimuli contact the apical region of the TRC by way of a taste pore. Taste buds also contain support cells that do not have taste receptors. Some support cells are immature TRCs, while others are thought to provide structural support to the receptor cells (Kinnamon, 1987). The majority of taste

buds are found on the tongue in distinct epithelial structures called papillae and in the soft palate. In the rat, fungiform, or mushroom-shaped papillae, are located on the anterior two-thirds of the tongue, and a single, round circumvallate papilla can be found on the posterior surface of the tongue. Foliate papillae resembling gills are located on the sides of the tongue toward the back. A small number of taste buds can also be found on the larynx, epiglottis, nasoincisor ducts and esophagus. In the rat, the circumvallate papilla contains considerably more taste buds than the other papillary fields (~ 60% of the total number). The fungiform papillae contain approximately 15%, the palate 15%, and the remaining 5-10% are distributed among the other gustatory fields (Miller, 1995; Travers & Nicklas, 1990). Individual variance commonly exists both in the number of papillae per anatomic region and the number of taste buds per papilla (Miller, 1995).

Dendrites from a single axon can synapse with cells from more than one taste bud, including buds located in different papillae. It is unclear whether buds that synapse with the same nerve fiber express the same receptor proteins. This system would seem to suggest convergence of information; however, individual taste buds can also be innervated by more than one axon, suggesting that peripheral coding has the potential to be somewhat more complex than it initially appears. In addition, TRCs are replaced every 10 days (Beidler & Smallman, 1965). In spite of this, response profiles from the nerves remain remarkably stable. It has been proposed that soluble factors released by the nerve or by adjacent cells affect the development of the new cell (see Miller, 1995). The number and variety of receptors found on each TRC are also a source of debate. Intracellular recording techniques have shown that most TRCs respond to a number of

stimulus classes, suggesting that most, but not all, cells contain several different types of taste receptors (Gilbertson et al., 2001).

When a stimulus interacts with a taste receptor, the TRC undergoes a change in membrane potential or intracellular calcium concentration leading to neurotransmitter release (see Herness & Gilbertson, 1999). Hence, the TRCs form chemical synapses with the innervating dendrites. When the TRC releases enough neurotransmitter, an action potential is produced in the innervating neuron. In addition to transmitting taste information to the peripheral nerves, these neurotransmitters might also bind to other TRCs in the taste bud, modifying their activity. Neurotransmitters associated with TRCs to date include serotonin, GABA and norepinephrine (Herness & Gilbertson, 1999). The presence of GABA suggests that inhibition as well as excitation may provide meaningful gustatory information. Peptides also thought to be released by TRCs with stimulation include bombesin, cholescystokinin, histidine, neuropeptide Y, and somatostatin (Norgren, 1995). Cells in a taste bud might also communicate with one another by way of gap junctions (Holland et al., 1989). Such a mechanism could lead to the excitation of adjacent cells without the use of neurotransmitters. Because not all TRCs synapse with taste afferents, electrical coupling could potentially allow information to be transmitted from a greater number of cells than chemical transmission alone (Herness & Gilbertson, 1999).

Gustatory Nerves

Rats have 3 main nerves that carry taste input. The chorda tympani (CT) branch of the facial nerve, or cranial nerve VII (CN VII), innervates the taste buds in the anterior two-thirds of the tongue (i.e., the fungiform papillae). The greater superficial petrosal (GSP) branch of this nerve innervates taste buds in the palate and nasoincisor ducts and the

glossopharyngeal nerve (GL), or CN IX, carries taste information from taste buds located in the circumvallate papilla and a portion of the foliate papillae. The CT innervates the remainder of the taste buds in the foliate papillae. The superior laryngeal (SLN) branch of the vagus nerve, or CN X, also synapses with taste buds. These are located in the epiglottis, esophagus and larynx and are thought to be more important for airway protection than for the perception of taste quality (Dickman & Smith, 1988; St John & Spector, 1998; Smith & Hanamori, 1991). The somata of these gustatory cranial nerves are located in 3 ganglia. The CT and GSP somata reside in the geniculate ganglion. Glossopharyngeal somata are found in the inferior petrosal ganglion, and the cell bodies of the SLN are in the inferior nodose ganglion (Miller, 1995). These ganglia also provide some parasympathetic innervation to salivary glands (see Smith et al., 1988).

In addition to differences in the receptor fields innervated by these nerves, the nerves also differ in response profile. For instance, electrophysiology has shown that the CT contains 2 classes of fibers, one that is selective for sodium and lithium salts and one that is more broadly-tuned (Frank et al., 1983). This second class of fibers is highly responsive to salts and acids and more moderately responsive to alkaloids like quinine. The GSP is highly responsive to sugars while moderately responsive to salts, acids and quinine (Nejad, 1986; Sollars & Hill, 1998). The GL contains 3 classes of fibers; one class that responds best to salts and acids, one that responds best to sugars, and one that is highly responsive to alkaloids (Boudreau et al., 1983; Frank, 1991). Fibers from the SLN do not seem to form units based on chemical sensitivity but are highly responsive to stimuli described as “sour” or “bitter” by humans (Dickman & Smith, 1988).

Central Gustatory System

Medullary and Pontine Taste Nuclei

Second-order neurons of the gustatory system reside in the lateral region of the rostral nucleus of the solitary tract (NST) located in the medulla. Terminal branches from the peripheral nerves are distributed such that the nerves innervating the most rostral part of the oral cavity (i.e., the CT and GSP) synapse most rostrally in the gustatory NST. The GL is more caudally represented and the vagus nerve more caudal still. In spite of this general division, however, there is still a large degree of overlap in the terminal fields of the 3 main gustatory nerves (Hamilton & Norgren, 1984; Travers & Norgren, 1995). Trigeminal fibers also terminate in the lateral portion of the rostral NST while visceral projections from vagal afferents terminate in the caudal NST (Finger, 1987; Travers, 2002). The majority of axons from neurons in the gustatory NST project to the ventromedial subnucleus of the parabrachial complex, henceforth referred to as the parabrachial nucleus (PBN). This nucleus is located in the dorsal pons and receives both gustatory and visceral input. These inputs are mostly, but not entirely, segregated (Hermann & Rogers, 1985). Apart from the PBN, some gustatory neurons in the rostral NST project to the parvicellular reticular formation where they synapse with parasympathetic salivatory neurons and cells from the hypoglossal nucleus (see Halsell et al., 1996; Norgren, 1995). These pathways are thought to be responsible for reflexive oromotor responses to taste stimuli (see Travers & Norgren, 1983). In primates, most projections from the gustatory NST bypass the PBN and synapse directly onto thalamic neurons (Beckstead et al., 1980).

Ascending Gustatory Pathways

From the PBN, input from taste nerves is channeled into 2 separate functional pathways. The lemniscal or thalamocortical gustatory pathway projects bilaterally in the parvocellular region of the ventral posteromedial nucleus (VPMpc) of the thalamus before reaching the cortex (Kosar et al., 1986; Norgren & Leonard, 1973). Although this pathway is often touted as being responsible for learned taste associations, there is evidence to the contrary. For instance, ibotenic acid lesions of the gustatory thalamus fail to impair conditioned taste aversion acquisition (Flynn et al., 1991; Reilly, 1998). In addition, both the amygdala and PBN send some projections directly to the gustatory cortex, which could be necessary for sensory discrimination, although it is not clear whether these fibers carry information regarding taste quality. The ventral gustatory pathway, or pontolimbic system, projects mainly from the PBN to the amygdala, but also projects to the lateral hypothalamus, bed nucleus of the stria terminalis and other limbic regions of the forebrain (Norgren, 1995). This pathway is thought to be important for the hedonic responses to taste stimuli.

In addition to gustatory projections from the hindbrain to the forebrain, telencephalic structures also project back onto the thalamus, PBN, NST, amygdala and hypothalamus. The amygdala and hypothalamus also send axons to the PBN and NST and the PBN reciprocally innervates the NST. These projections from limbic and cortical areas could lead to feedback onto taste pathways from other sensory modalities, as well as from areas involved with motivation and emotion (see Norgren, 1995). Recent evidence supporting this hypothesis has shown that the tuning characteristics of taste-responsive neurons in the PBN are altered with stimulation of either the lateral hypothalamus or central amygdala (Cho et al., 2002; Lundy & Norgren, 2001).

The NST appears to be necessary for rats to respond to the taste quality of a stimulus. When this area is lesioned, concentration-response functions become flattened for all 4 prototypical stimuli (Shimura et al., 1997). While the NST appears to be necessary for the perception of taste quality, it has been suggested that the role of the PBN in taste processing involves the integration of taste quality with visceral input (see Grigson et al., 1998; Spector et al., 1992). For example, after bilateral PBNX a rat is unable to acquire a conditioned avoidance response, a sodium appetite or display successive negative contrast (Flynn et al., 1991; Grigson et al., 1994; Grigson et al., 1998; Scalera et al., 1995), but can still form associations between 2 tastants or between a somatosensory cue and malaise (Grigson et al., 1998; Reilly et al., 1993). Expression of a conditioned taste aversion acquired prior to PBN lesion does not seem to be affected by the surgery (Grigson et al., 1997).

Lesions of the gustatory thalamus and cortex appear to have little or no effect on innate taste processing in the rat (Reilly & Pritchard, 1996), but may affect the acquisition and/or retention of a conditioned taste aversion (see Spector et al., 1992 for review). Grigson and colleagues (1998) have proposed that in addition to the NST and PBN, at least one forebrain structure, such as the amygdala or thalamus, must be intact to elicit conditioning to a taste stimulus. Electrophysiological recordings from neurons in the gustatory cortex have suggested that these cells might respond to the hedonic characteristics of the taste stimulus rather than to its associated taste quality (Yamamoto et al., 1989). Recent evidence using chronic recording techniques suggests that taste quality might be discerned from temporal patterns at this level recorded between 0.2 and 1 s after stimulus onset (Katz et al., 2001).

Taste Transduction

Primary Tastes

There are 4 prototypical classes of tastants; “salty,” “sweet,” “bitter” and “sour.” A fifth taste known as “umami” that has been described as being “meaty” or “savory” is gaining acceptance among taste researchers and is typified by the taste of monosodium glutamate, or MSG. Umami compounds often modify the taste qualities of other compounds in addition to producing a taste themselves. It should be noted, however, that these categories are not definitive as they are based on the taste quality most associated with a particular stimulus, or set of stimuli, by human subjects and often only within a narrow range of effective concentrations. Sodium chloride, although often used as the prototypical “salty” stimulus in taste research, is said to taste “sweet” at very low concentrations (Bartoshuk et al., 1978), indicating that a stimulus may produce more than one taste quality depending on concentration. In addition, this salt has also been described as “sour/salty” at moderate concentrations, suggesting that even a prototypical stimulus like NaCl can produce significant side-band tastes (van der Klaauw & Smith, 1995). The fact that these taste qualities are based on human data also casts doubt on whether these 5 categories, or their prototypical stimuli, are stable across species. Other possible taste categories include “electric,” “fatty acid” and “water” although much less is known about the perceptual and physiological properties of these sensations than the others mentioned here (see Gilbertson et al., 1997; Ninomiya & Funakoshi, 1989; Shingai, 1980). The prototypical classes of tastants can be divided into 2 groups based on transduction mechanism.

Transduction Mechanisms

Ion channels

Salt and acid stimuli dissociate in the saliva releasing free protons in the case of acids, and ions in the case of salts that pass through ion channels in the TRC membrane. Epithelial sodium channels (ENaCs), probably the best-characterized ion channels in the oral cavity of the rat, are voltage-sensitive and highly selective for sodium and lithium ions (see Garty & Palmer, 1997; Ye et al., 1991). Less selective, voltage-insensitive ion channels also allow sodium ions to pass into the cell (Ye et al., 1991). Acid transduction mechanisms fall into 3 main categories. Protons can pass into the TRC as a result of the concentration gradient, as observed in the hamster (Gilbertson et al., 1992), block cation channels, as observed in the frog and mudpuppy (see Kinnamon & Margolskee, 1996) or bind to sites on the basolateral membrane of TRCs (DeSimone et al., 1995).

Metabotropic receptors

Stimuli giving rise to "sweet," "bitter" and "umami" sensations in humans have been shown to activate G-protein-coupled receptors in the oral cavity (see Kinnamon & Margolskee, 1996). A variety of second messenger pathways are reportedly affected by this coupling, including cAMP, inositol triphophate (IP_3) and diacyl glyceride (DAG). Both cAMP and IP_3 pathways are activated by stimuli that produce "bitter" or "sweet" taste qualities. Artificial sweeteners and amino acids are thought to use the IP_3 pathway, while natural sugars are thought to use a cAMP-dependent pathway (Herness & Gilbertson, 1999).

Unfortunately, there is not a one-to-one correspondence between taste qualities and transduction pathways. For example, 2 stimuli with different taste qualities can share a common route of transduction. In the hamster, although both salts and acids use the same

amiloride-sensitive pathway (Gilbertson et al., 1992), these stimuli result in different qualitative perceptions (Nowlis et al., 1980). In addition, stimuli that share a common taste quality like quinine and denatonium, stimuli described as "bitter" by humans, can affect more than one transduction pathways. Quinine is thought to be cAMP-dependent and denatonium uses an IP_3 second messenger pathway (Herness & Gilbertson, 1999), yet these 2 compounds produce a unified taste perception (Spector & Kopka, 2002). A single stimulus can also potentially activate more than one transduction pathway. Sodium ions activate 2 transduction pathways in the rat, but only one of these appears to be important for producing the characteristic taste quality associated with sodium.

Sodium Transduction Pathways

Amiloride-sensitive (AS) pathway

The amiloride-sensitive transduction pathway in the oral cavity of the rat is selective for sodium and lithium ions (Brand et al., 1985; DeSimone & Ferrell, 1985) and activation of this pathway is significantly reduced with application of the ENaC blocker amiloride (DeSimone & Ferrell, 1985; Ninomiya & Funakoshi, 1988). Thus, functional amiloride-sensitive sodium channels, or ASSCs, are thought to be located in the apical region of taste receptor cells (DeSimone & Ferrell, 1985). Immunohistochemistry has also indicated that ASSCs are also located in the basal region of some TRCs, but these appear to be nonfunctional under normal circumstances (Lin et al., 1999). Sodium responses are only suppressed by amiloride in the CT and GSP nerves, suggesting that TRCs with functional ASSCs are predominantly, if not exclusively, innervated by the gustatory branches of the facial nerve (Kitada et al., 1998; Ninomiya & Funakoshi, 1988; Sollars & Hill, 1998).

When a rat is depleted of body sodium, it will ingest increased quantities of sodium-containing solutions. This phenomenon is called sodium appetite (see Denton, 1982; Schulkin, 1991). With amiloride treatment both the magnitude and specificity of a sodium appetite are diminished (Bernstein & Hennessy, 1987). Amiloride treatment also impairs the rat's ability to discriminate between NaCl and KCl (Spector et al., 1996). Together, these studies suggest that the amiloride-sensitive pathway is necessary for the rat to perceive the taste quality of sodium. While amiloride appears to be tasteless to the rat (Hill et al., 1990; Markison & Spector, 1995), humans report that it possesses a "bitter" quality (Schiffmann et al., 1983). Studies using human subjects also indicate that although amiloride decreases the perceived intensity of sodium salts, this decrease is observed only in the "sour" component of the stimulus, leaving "saltiness" unaffected (Ossebaard & Smith, 1995).

Amiloride-insensitive (AI) pathway

The amiloride-insensitive pathway, on the other hand, is permeable to a variety of ions including Na^+, Li^+, K^+ and NH_4^+. This pathway also appears to use receptors located in the basolateral region of the TRC. For this reason, ions are thought to pass through tight junctions between cells before accessing receptor channels. Therefore, these ions must have a small radius. Amiloride is thought to be too large to fit through these tight junctions, making this pathway insensitive to the sodium channel blocker. Much less is known about this pathway than about the AS pathway as it is much more difficult to access. Although it does not appear to be affected by amiloride, transduction via this pathway can be partially blocked by introducing salts with large anions. Sodium passage through tight junctions depends on the electroneutral diffusion of ions. If the anion is too large to fit through these junctions, the cation will also fail to reach the receptor sites due

to the unfavorable electrical gradient. In support of this hypothesis, the CT nerve is much less responsive to sodium gluconate (NaG) and other large anion sodium salts than to NaCl at the same concentration (Formaker & Hill, 1988; Ye et al., 1993), suggesting that perhaps the large gluconate ion keeps the sodium ions from passing through the tight junctions. This effect does not seem to be due to a loss of Cl^- receptor conductance, as Cl^- channel antagonists do not affect CT responses to NaCl (Elliot & Simon, 1990). Thus, the response to NaG appears to depend almost entirely on activation of the amiloride-sensitive pathway. Interestingly, the detectability functions for NaCl and NaG are strikingly similar (Geran & Spector, 2000b), suggesting that the AS pathway is not only necessary for sodium detection but also sufficient. It could be argued that this necessity and sufficiency qualify this pathway as a labeled line for sodium taste in the rat.

Labeled Line vs. Across-Fiber Pattern Theory

Briefly, labeled line theory states that activation of a particular set of neurons leads to the perception of a particular quality. When the activation is below a certain threshold, the quality is not perceived and when activation reaches this threshold, it is perceived. The across-fiber pattern (AFP) theory states that the overall pattern produced by the presence and absence of activation across all afferent taste fibers or central neurons produces the perceived taste quality (see Pfaffman, 1959). In the strictest sense, a labeled line should be both necessary and sufficient to produce a given perception. Most of the data supporting the AFP theory come from the fact that gustatory neurons at each level of the neuraxis respond to several tastant classes. How much of this activity is “signal” and how much is “noise” is a matter of debate.

The breadth of tuning of neurons in the NST is often used to support the AFP theory. Although NST neurons are responsive to a variety of taste stimuli, 2 lines of evidence

suggest that perhaps the activity of these neurons is more selective than previously thought. The first such evidence comes from immunohistochemistry. Experiments conducted by Travers and colleagues have shown that the immediate early gene c-Fos is differentially expressed in the NST in response to sucrose, citric acid and quinine (Harrer & Travers, 1996; Travers, 2002). Although overlap exists for the three stimuli, these data suggest that there may be some chemotopic arrangement within the gustatory regions of the CNS, although more experiments are required. The second line of evidence comes from electrophysiological examinations of higher brain regions in the gustatory neuraxis using either awake animals (Nakamura & Norgren, 1993) or animals in which the amygdala, gustatory cortex or lateral hypothalamus was stimulated in conjunction with tastant presentation (see Cho et al., 2002; Di Lorenzo & Monroe, 1995, Lundy & Norgren, 2001). Under these conditions, NST neurons are less broadly-tuned than in an anesthetized preparation in the absence of electrical stimulation. Hence, a feedback mechanism exists through which medullary and pontine taste responses could potentially be modified according to the motivational state or previous experience of the animal.

If one takes a looser approach to the two theories they are not necessarily mutually exclusive. For instance, it is possible that one theory holds for some stimuli but not for others. For example, much of the behavioral evidence suggests that sodium taste might utilize a labeled line system, although the perception of other tastes might require activation of several fiber types or transduction mechanisms. Labeled lines have also been hypothesized to account for “sweet” taste in the hamster and chimpanzee (Danilova et al., 1998; Hellekant et al., 1998). It is also possible that the behavioral task in question might be more parsimoniously described by one theory than by the other. For instance,

recognition or detection tasks might only require activation of one pathway for normal performance, while a discrimination task might require the animal to perceive more subtle differences in activity that might best be described in terms of AFP theory.

Currently, it appears that AFP theory has become the accepted paradigm, although it can be argued that a somewhat labeled line-like approach has become pervasive in the form of "best stimulus" categories. Gustatory neurons are often classified according to the prototypical stimulus that produces the greatest magnitude of responding. For instance, a neuron that responds highly to sucrose, moderately to NaCl, somewhat to citric acid and only marginally to quinine would be termed a "sucrose best" cell. Activity from each of the cells in this category would be then be averaged and compared to the mean activity of other "best stimulus" categories. This method could potentially keep more subtle patterns of activity, perhaps due to second or third-best stimulus categories within a "best stimulus" classification from becoming apparent. In a strict across-fiber pattern code excitation of all cells regardless of best stimulus, as well as inactivity and inhibition of neurons with gustatory input, could potentially be meaningful and should therefore be analyzed. Another caveat to this method of analysis is the fact that researchers do not record from each cell in a particular anatomical region. Recording from every cell in the NST would, of course, be a herculean task, but recording only from those that are most accessible could potentially lead to a skewed sample. While tests of necessity and sufficiency can be performed at some levels of the gustatory system to shed light on the AFP vs. labeled line debate, we are not currently able to block responses from a particular best-stimulus category of the NST so that the same tenets might be applied to responses in the CNS. In spite of these caveats, predictions based on AFP

theory appear to correlate well, for the most part, with the results of stimulus generalization tasks (see Erickson, 1963). As mentioned previously, gustatory coding is most likely best explained by some amalgamation of the 2 competing theories. The following chapters will examine the necessity and sufficiency of the AS and AI salt transduction pathways for a variety of taste-guided tasks. Whether these data support or challenge the existing coding theories will also be addressed.

CHAPTER 2
ANION SIZE DOES NOT COMPROMISE SODIUM RECOGNITION BY RATS FOLLOWING ACUTE SODIUM DEPLETION

Background

When rats are in a sodium-depleted state, the apparent perception of a "sodium-like" taste quality will promote ingestion of the stimulus (e.g., Falk & Herman, 1961; Handal, 1965; Nachman, 1962; Richter & Eckert, 1938). This depletion-induced elevation of intake, termed sodium appetite, is specific for sodium and lithium salts (Nachman, 1962) and has been described in a number of mammals (see Denton, 1982). Researchers have used this phenomenon for several decades to test hypotheses about salt taste perception, most notably through the use of brief-access taste tests which substantially reduce the contribution of postingestive receptors (e.g., Breslin, et al., 1993; Falk & Herman, 1961; Handal, 1965; Nachman, 1962).

The ability to recognize the taste of sodium when in a sodium-depleted state appears to depend upon taste receptor cells in the oral cavity that contain ion channels selective for sodium and lithium ions (Bernstein & Hennessy, 1987; McCutcheon, 1991; Roitman & Bernstein, 1999). The sodium-selective ion channels, or epithelial sodium channels (ENaCs), expressed by these cells can be blocked with the drug amiloride (see Brand et al., 1985; DeSimone & Ferrell, 1985; Doolin & Gilbertson, 1993; Heck et al., 1984; Schiffman et al., 1983). A second transduction pathway for sodium, the amiloride-insensitive (AI) pathway is not sodium-selective, but instead appears to be activated by a variety of cations including Na^+, K^+, and NH_4^+ (Brand et al., 1985; DeSimone & Ferrell,

1985; Kloub et al., 1997;Ye et al., 1994). Instead of ions passing directly through apical ENaCs, activation of this pathway is thought to involve the diffusion of ions across tight junctions and also perhaps through less selective ion channels in the apical membrane (DeSimone & Ferrell, 1985; DeSimone et al., 2001; Elliot & Simon, 1990; Simon, 1992; Ye et al., 1993; 1994). Activation of the AI pathway can be significantly reduced by pairing the cation with an anion of large hydrated radius, like acetate or gluconate, thus reducing the diffusion of ions across tight junctions (Elliot & Simon, 1990; Rehnberg et al., 1993; Simon, 1992; Ye et al., 1993). Although recent data support the existence of an amiloride-insensitive, nonselective cation channel in the apical membrane (DeSimone et al., 2001; Gilbertson & Zhang, 1998), large anion salts apparently do not significantly stimulate this pathway. Potassium gluconate, for example, is a very poor stimulus (Stewart et al., 1996; Ye et al., 1994) and amiloride treatment virtually eliminates the CT response to sodium acetate (NaAc) and sodium gluconate (NaGlu), especially at low to mid-range concentrations (Elliot & Simon, 1990; Formaker & Hill, 1988; Ye et al., 1993).

Previously, it has been shown that the amiloride-sensitive (AS) pathway is both necessary and sufficient for normal Na^+ detection in the rat, at least to the extent that gluconate is capable of blocking AI transduction (Geran & Spector, 2000a; 2000b). It is possible, however, that these near threshold concentrations, although detectable, were not perceived as tasting "sodium-like" by the animals. For instance, humans often report that low concentrations of NaCl taste "sweet" rather than "salty" (Bartoshuk et al., 1978). Consequently, the effect on sodium recognition of reducing the contribution of the AI pathway(s) with a large anion was tested. Other researchers have shown that rats will

ingest a variety of sodium salts when depleted, but to our knowledge all have used single stimulus (Handal, 1965; Krieckhaus & Wolf, 1968; Morrison & Young, 1971), 24-h intake (Fregly, 1958; Richter & Eckert, 1938), or 2-bottle sodium vs. nonsodium salt tests (Nachman, 1962), making comparisons among taste-guided preferences for different sodium salts impossible. Instead, we used a brief-access test so that preference for several salts could be analyzed simultaneously and without potentially confounding postingestive effects. Three sodium salts with different sized anions were used to limit AI transduction (Elliot & Simon, 1990; Formaker & Hill, 1988; Kitada et al., 1998; Ye et al., 1993). Sodium gluconate in particular was chosen based on its ineffectiveness in stimulating AI transduction (Ye et al., 1991; 1993). This experiment marks the first time to our knowledge that this salt has been presented to sodium-depleted rats. Two concentrations of each salt were chosen on the basis of mean sodium detectability functions measured previously (Geran & Spector, 2000a; 2000b). One concentration was well above detection threshold in the presence of amiloride (0.3 M), and the other below threshold when mixed with amiloride, but above threshold in the absence of amiloride (0.03 M). Potassium chloride and NH_4Cl were included in the stimulus array for the purpose of comparison. If the AS pathway is sufficient as well as necessary for sodium recognition, sodium-depleted animals should show similar amounts of licking to all sodium salts regardless of anion. Furthermore, this increase in licking to sodium salts should be abolished with the addition of amiloride.

Methods

Subjects

A total of 40 naïve male Sprague-Dawley rats (Charles River Breeders, Wilmington, MA) were used in this experiment. Subjects were tested in 2 groups of 20. All rats

weighed approximately 250-300g at the start of training and were placed on a 12:12 light:dark schedule with lights on at 6:00 AM. Humidity and temperature were kept constant. Rats were individually housed in hanging wire mesh cages and given *ad libitum* access to laboratory chow (PMI 5001 pellets. PMI Nutrition International, Brentwood, MO) except where noted. Access to distilled water, however, was restricted while in the home cage. Water bottles were removed ~24 h prior to training and replaced after the last training session 5 days later. Rats had access to water only during 30-40 min training sessions on these 5 days. Body weights were closely monitored for excessive dehydration. No rat dropped below 85% of its individual *ad libitum* weight during this experiment. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida.

Apparatus

All training and testing occurred in a modified, automated taste testing apparatus called a gustometer (Spector, Andrews-Labinski, & Letterio, 1990). This apparatus was designed to deliver small volumes of fluid stimuli and record the number of licks for each stimulus presented. A narrow slot in one wall of the chamber enabled the rat to access a vertically-oriented sample spout. This spout rotated into position in front of the slot at appropriate times throughout the session. Each taste stimulus was kept in a pressurized syringe connected to the sample spout by way of Teflon® tubing and a solenoid valve. Each valve was opened for a preset amount of time by the computer such that each lick delivered ~5 μl of fluid after the drinking shaft was initially loaded with the stimulus (see Spector, Andrews-Labinski, & Letterio, 1990 for more details on stimulus delivery). A very low current (< 50 nA) contact circuit was used to monitor number of licks taken.

Training Procedure

On the first day of training, a drop of water was placed on the tip of the spout and on the inside wall of the chamber before each session to help in training the rat to drink from the spout. During the first 2 days of training the sample spout remained motionless in front of the access slot so that the rat had continuous access to distilled water for each 30-min session. After all animals had been trained to lick from the spout, they received 3 days of additional spout training in which they received access to distilled water and 0.1 M sucrose in 5s trials. Sucrose was used to encourage licking. The rat was required to lick the dry spout twice in 1 s to receive a stimulus presentation. When the trial was finished, the spout rotated away from the access slot and over a funnel where it was rinsed with distilled water and dried with pressurized air. This process took about 6s to complete. The spout then rotated back in front of the slot. These sessions lasted 40 min.

Sodium Depletion

After training was complete, rats were assigned to one of 4 groups. These groups were counterbalanced for body weight, number of trials initiated during the last 3 days of training and mean number of total licks for these 3 days. Distilled water bottles were placed on the home cages Friday following the last training session. On Monday morning the rats were moved from their standard cages to metabolism cages equipped with funnels to collect urine. They were also given a weighed amount of powdered chow at this time instead of pellets. Rats in the 2 sodium-depleted groups received Harlan Teklad 90228 sodium-deficient (0.02% NaCl) chow (Harlan Teklad, Madison, WI). Rats in the 2 non-depleted groups received the same chow mixed with 1.0% NaCl. Twenty-four hours prior to testing, each rat in the sodium-depleted groups received the first of 2 equal volumes of furosemide (total dose = 30mg/kg BW, s.c.). The second furosemide injection

was given 2 hours later. Rats in the non-depleted groups received injections of isotonic saline (s.c.) using the same injection schedule and volume as rats given furosemide. Subjects had free access to powdered chow (with or without sodium) and distilled water during the sodium depletion phase of the experiment. Urine was collected in 100 mL flasks for 24 h immediately following the first injection.

Brief-Access Testing

Testing took place in the gustometers 24 h after each animal's first furosemide injection. Animals were given brief access to 11 stimuli (e.g. distilled water and 0.03 & 0.3 M concentrations of NaCl, KCl, NH_4Cl, sodium acetate (NaAc), and sodium gluconate (NaGlu)) over a 40-min period. Stimuli were presented in randomized blocks except that the first trial of each session was always 0.3 M NaCl to encourage continued sampling. All salt solutions were made fresh using reagent grade chemicals (all salts from Fisher Scientific, Orlando, FL except NaGlu from Sigma Chemical Co., St. Louis, MO) and distilled water. One liter of 100 μM amiloride (Sigma Chemical Co.) stock solution was made the evening prior to testing and wrapped in aluminum foil and left on a stir pad overnight in a dark room. Two of the 4 gustometers contained the aforementioned salts dissolved in distilled water, while the remaining 2 gustometers contained salts dissolved in 100 μM amiloride and 100 μM amiloride in place of the distilled water stimulus. Half of the rats from each depletion group were tested with amiloride as the solvent.

At the time of testing, the remaining chow was removed from the home cage and weighed to determine how much was ingested in the previous 24 h after attempting to account for spillage. Distilled water intake and urine output for each rat were measured to

the nearest mL. A 2.0 mL sample of urine was collected for each rat and frozen in labeled plastic centrifuge tubes for later analysis.

Urine Analysis

Urine was analyzed using a flame photometer to determine sodium content for both sodium-depleted and non-depleted rats. Urine from sodium-depleted rats was diluted with distilled water (4 parts water: 1 part urine) prior to analysis so that the sodium concentration of each sample would fall within the range testable by the device. The values for these animals were then multiplied by a factor of 5.

Data Analysis

Lick data were recorded and analyzed for the entire 5s of each trial, but the main parameter of interest was the number of licks to each stimulus during the last 3s of each 5s trial (i.e., the avoidance period). This was done to minimize the number of sampling licks included in the analysis. The local lick rate for rats is approximately 7 Hz (Corbit & Luschei, 1969; Halpern, 1977), making 35 licks the ceiling in a 5s trial and 21 licks the highest performance attainable in a 3s period. The number of licks for each stimulus was then averaged for each group and compared using analyses of variance (ANOVAs) and *t*-tests (paired, independent and one-sample tests). The statistical rejection criterion was set at .05 for all analyses. P-values were adjusted using the Bonferroni method when a large number of t-tests were performed on the same data set. Lick data from an animal were included in the analyses only if the animal sampled all 11 stimuli in the test. Only 2 rats in the non-depleted/amiloride group and 1 rat in the non-depleted/water group passed this criterion. This sample size was too low for meaningful analysis, so all statistical tests of stimulus licking were performed on sodium-depleted rats only. Data from each rat were used, however, to analyze the number of trials initiated during testing and degree of

sodium depletion for each of the 4 groups. Sodium balance was determined by subtracting urinary sodium output from sodium ingested.

Results

Brief-Access Testing

The overall pattern of responsiveness for the sodium-depleted groups was the same regardless of whether the 3s avoidance period or the entire 5s period was analyzed. One-way ANOVAs of mean licks during the last 3s of each trial revealed a main effect of stimulus for both the amiloride ($F(10, 90) = 35.4$, $p < .001$) and distilled water ($F(10,90) = 2.7$, $p < .008$) groups. Intake of distilled water and amiloride did not differ significantly between groups ($p > .09$, 10.3 ± 4 vs. 13.6 ± 4 licks respectively).

For rats in the distilled water condition, paired t-tests indicated that they licked significantly more to each of the 6 sodium stimuli (i.e. 3 salts, 2 concentrations) than to water ($p < .005$ for each t-test. Bonferroni adjusted $p < .05$. Figure 2-1). Furthermore, a 2-way ANOVA (anion x concentration) of responses to sodium salts revealed a significant main effect for concentration only ($F(1,9) = 26.9$, $p < .002$). One-way ANOVAs indicated no differences in performance across sodium salts at the 0.03 M concentration and a slight effect at the 0.3 M concentration ($F(2,18) = 3.99$, $p < .04$), with the greatest number of licks recorded for the salt with the intermediate-sized anion, sodium acetate (NaAc). Paired t-tests indicated significantly less licking to the NH_4Cl solutions than to water ($p < .008$ for both) but this significance disappeared with a Bonferroni test (adjusted $ps > .07$). The KCl stimuli were not different from water ($p > .34$ for both, unadjusted).

In the amiloride condition, .03 M NaGlu and .3 M NH_4Cl were licked less than amiloride alone ($p < .05$ for both). Again, the statistical significance of this difference

disappeared with a more conservative test (adjusted ps > .4). All other stimuli generated licking comparable to that induced by amiloride alone (all ps > .14, unadjusted). A two-way ANOVA (anion x concentration) for all sodium salts indicated that all 6 sodium stimuli were licked to similar degrees in the presence of amiloride (ps > .14).

Number of Trials Initiated

Not surprisingly, the animals in the sodium-depleted groups initiated a greater number of trials than rats in the non-depleted groups ($p < .001$). However, sodium-depleted rats in the amiloride condition took far fewer trials than sodium-depleted rats in the distilled water condition ($p < .001$, Figure 2-2). This suggests that although more motivated to lick than non-depleted rats, the presence of amiloride reduced the number of trials initiated by sodium-depleted rats.

Sodium Balance

All rats in the sodium-depleted groups were in negative sodium balance while rats in the non-depleted groups ingested more sodium than they excreted ($p < .004$ for each of the 4 groups. Figure 2-3). There was no difference in sodium balance between rats in the distilled water condition and those in the amiloride condition for either depletion group (both ps > .20).

Discussion

It is clear from the data that the sodium-specific appetite exhibited by rats in the distilled water condition was not evident in the amiloride condition. When amiloride was added to the taste stimuli, intake of the sodium salts was not greater than intake of either the nonsodium salts NH_4Cl and KCl or of 100 μM amiloride alone, strongly suggesting that the animals were unable to recognize the sodium salts and to ingest them preferentially. These findings support previous reports of impaired sodium appetite with

amiloride (Bernstein & Hennessy, 1987; McCutcheon, 1991; Roitman & Bernstein, 1999), and extend them by showing that the taste-guided specificity of the sodium appetite is completely abolished by amiloride treatment in a brief-access test.

This effect of amiloride on the specificity of the sodium appetite differentiates amiloride treatment from CT transection, which has been shown to impair but not always eradicate sodium-specific appetite (see Breslin et al., 1993; Markison et al., 1995). This difference is likely due to the fact that the greater superficial petrosal (GSP) branch of the facial nerve also contains amiloride-sensitive fibers important for the maintenance of certain taste-guided tasks involving sodium (Roitman & Bernstein, 1999; Sollars & Hill, 1998). Our results also differ from those of CT transection in certain other regards. For instance, prior studies reported increased licking to low KCl concentrations with transection (Breslin et al., 1993) that we did not observe with amiloride treatment. This is most likely due to the fact that CT transection reduces the perceived intensity of KCl in rats while amiloride does not (Geran et al., 1999). Procedural differences between this experiment and the brief-access studies with CT transection should also be taken into account when comparing performance between experiments. These include a shorter sample time (5s vs. 10s), a greater sodium : nonsodium salt ratio in the stimulus array (3:2 vs. 1:4) and a slightly lower (0.03 vs 0.05 M) concentration of KCl in the current experiment.

Also of importance is the fact that sodium-depleted rats in the distilled water condition increased intake of all sodium salts (NaCl, sodium acetate and sodium gluconate) to a similar degree. Although previous studies have reported licking to a variety of sodium salts following sodium depletion in rats (see Fregly, 1958; Handal, 1965; Nachman,

1962) the present study is the first sodium appetite experiment to our knowledge to include a variety of sodium salts in a single brief-access test. Our results strongly suggest that eliminating, or at least severely reducing, the contribution of the AI transduction pathway by changing the size of the anion does not compromise sodium recognition. Thus, the AS sodium transduction pathway appears to be both necessary and sufficient for sodium recognition in the rat just as it is for normal sodium detection. Evidence of an apically-located AI cation transduction pathway in the oral cavity of the rat (DeSimone et al., 2001; Gilbertson & Zhang, 1998) may call this sufficiency into question if this nonselective apical pathway is substantially activated by large anion sodium salts like NaGlu. However, current electrophysiology indicates that only a negligible portion of the CT response to NaAc and NaGlu remains with amiloride treatment at the stimulus concentrations used (Elliot & Simon, 1990; Formaker & Hill, 1988; Ye et al., 1993).

The functional role of the AI transduction pathway to taste function is yet to be understood, but might involve the detection and/or recognition of nonsodium salts. Support for this hypothesis comes from evidence that the discrimination of 2 nonsodium salts, NH_4Cl and KCl, is severely compromised by combined transection of the CT and GSP nerves but unaffected by amiloride treatment (Geran et al., 2002). This suggests that recognition and discrimination of these salts depends on amiloride-insensitive, salt-responsive units in these nerves. As noted above, normal KCl detectability is also compromised by CT transection but not significantly affected by amiloride treatment, suggesting that an AI pathway with input carried by the facial nerve is required for this task (Geran et al., 1999). Discrimination among sodium salts might also depend upon AI transduction. The CT response to NaCl is of greater magnitude than the response to NaAc

or NaGlu due to a larger amiloride-insensitive response (Elliot & Simon, 1990; Formaker & Hill, 1988; Ye et al., 1993). This comparative increase in AI response does not appear to confer any particular salience to NaCl over other sodium salts at near-threshold levels (Geran & Spector, 2000b), but it could affect the taste quality or intensity of sodium salts in rodents at superthreshold levels. Humans have rated NaCl as more intense than NaGlu at equimolar concentrations (Ossebaard & Smith, 1995). Recordings from the GSP, however, indicate roughly equivalent amiloride suppression to both NaCl and NaAc at concentrations of .1 M and higher, but at .05 M the response to NaCl is more suppressed (~75%) than the response to NaAc (~50%, Sollars & Hill, 1998). This suggests that AI sodium transduction in the GSP may be unaffected or even enhanced by larger anions. Alternatively, a third pathway might exist that is independent of sodium but responsive to acetate. Responses of the GSP to NaGlu have not been reported. Clearly, more research on the salt responsiveness of this nerve is needed.

Sodium-depleted rats in the distilled water condition increased their intake of both 0.03 and 0.3 M NaAc and NaGlu relative to water, suggesting that the size of the anion did not affect their ability to perceive the taste of sodium. In the amiloride condition, however, rats did not lick NaAc or NaGlu more than amiloride alone at either stimulus concentration, suggesting that sodium recognition was compromised. This is particularly noteworthy at the 0.3 M NaGlu concentration, due to the fact that rats were shown to detect this stimulus, albeit poorly, in the presence of amiloride (Geran & Spector, 2000b). Together, these results suggest that rather than responding to sodium activation of the AS transduction pathway, the behavioral detectability of high NaGlu concentrations derives from some cue related to the gluconate anion or perhaps activation of the AI pathway due

to leakage of sodium through tight junctions, or AI apical channels, as a result of the high Na^+ concentration gradient.

Sodium-depleted rats initiated a significantly greater number of trials than non-depleted rats regardless of whether amiloride was added to the stimuli. This indicates an increase in appetitive behavior (see Craig, 1918; Denton, 1982), even in the absence of a sodium taste cue. When the taste of sodium was present, sodium-depleted rats showed a further increase in the number of trials initiated. Thus, under these conditions, the taste of sodium in the absence of need does not produce an increase in appetitive behavior, while need in the absence of the appropriate taste cue does, although not to the same degree as need and gustatory cue combined.

In summary, these data support Bernstein & Hennessy's (1987) conclusion that the AS sodium transduction pathway is necessary for sodium recognition in the rat, and furthermore strongly suggest that this pathway is also sufficient (see Elliot & Simon, 1990; Formaker & Hill, 1988; Ye et al., 1993). We have also extended previous studies of the effects of amiloride on salt appetite to show that the sodium specificity of the appetite is completely abolished when the AS pathway is blocked. In addition, these findings suggest that although NaGlu concentrations higher than 0.1 M are detectable in the presence of amiloride they appear to lack the characteristic taste quality associated with sodium.

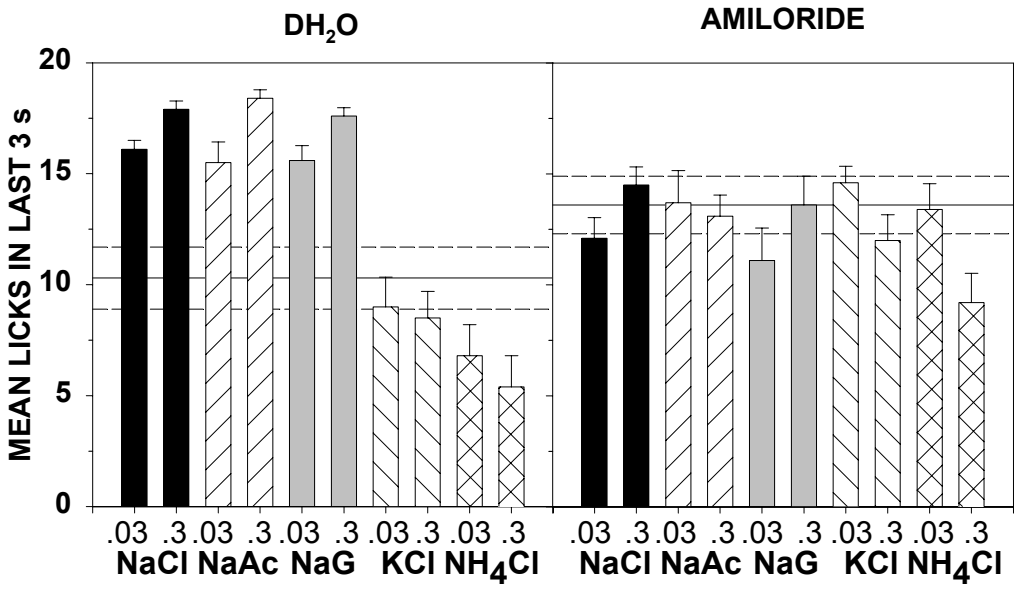

Figure 2-1. Brief-access licking to each stimulus by sodium-depleted rats. Mean (± SE) number of licks taken by sodium-depleted rats in the last 3s of each 5s bout. Stimuli were dissolved in either distilled water (left) or 100 μM amiloride (right). Horizontal lines indicate mean (± SE) number of licks to either distilled water or amiloride alone. All sodium salts (chloride, acetate and gluconate) were preferred over water (paired t-tests, $p < .005$ for each. $p < .05$ Bonferroni adjusted), while none of the salts, sodium or nonsodium (potassium chloride and ammonium chloride), were preferred over amiloride. Responses to water and amiloride alone were not significantly different.

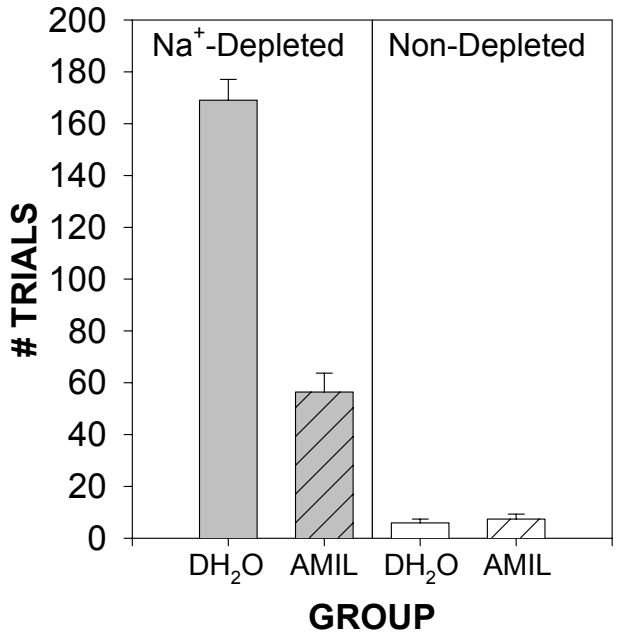

Figure 2-2. Mean (± SE) number of trials initiated by each group of rats. Non-depleted rats took the fewest trials regardless of whether amiloride was present. Sodium-depleted rats, however, initiated considerably fewer trials in the presence of amiloride ($p < .001$).

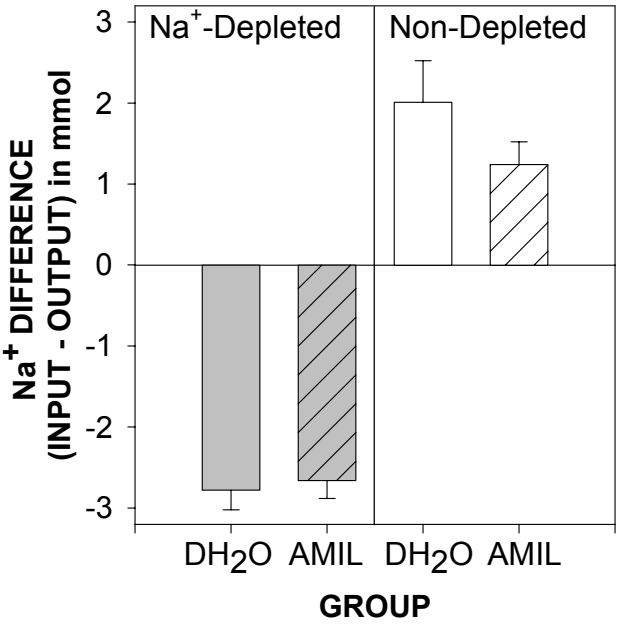

Figure 2-3. Mean (± SE) sodium balance for each group of animals measured in mmol. All rats in the sodium-depleted groups were in negative sodium balance prior to brief-access testing while rats in the non-depleted groups consumed more sodium than they had excreted ($p < .004$ for each group in a one-sample t-test).

CHAPTER 3
GLOSSOPHARYNGEAL NERVE TRANSECTION DOES NOT IMPAIR POTASSIUM CHLORIDE VS. AMMONIUM CHLORIDE OR SODIUM CHLORIDE VS. AMMONIUM CHLORIDE DISCRIMINATION

Background

Ammonium and potassium chloride have been shown to taste very similar to rats in generalization tasks (Erickson, 1963; Hill et al., 1990; Morrison, 1967). These results have been used to support both similarities in NH_4^+ and K^+ transduction at the receptor level and similarities in the neural response pattern at the level of the NTS (DeSimone et al., 2001; Erickson, 1963). Recently, it was reported that rats placed on a KCl vs. NH_4Cl discrimination task with overlapping concentrations were able to consistently perform well (~90% overall performance) after a typical period of discrimination training (Geran et al., 2002). Furthermore, dissolving the salt stimuli in the epithelial sodium channel (ENaC) blocker amiloride did not impair performance, but transecting the gustatory branches of the facial nerve (i.e., the chorda tympani (CT) and greater superficial petrosal (GSP) nerves) reduced performance to chance levels for each animal (Geran et al., 2002).

Rats trained and tested on a NaCl vs. NH_4Cl discrimination task also exhibited chance levels of performance following nerve transection even though the mean presurgical discrimination performance for this group was approximately 95% (Geran et al., 2002). Amiloride significantly impaired performance on this task, although not to the extent reported previously for NaCl vs. KCl discrimination (see Kopka et al., 2000; Spector et al., 1996). Sodium chloride vs. NH_4Cl discrimination was chosen as the comparison for KCl vs. NH_4Cl discrimination performance because rats do not generalize between NaCl

and NH_4Cl in a conditioned taste aversion task (Hill et al., 1990). This suggests that NaCl and NH_4Cl are easily discriminated by the rats, while KCl and NH_4Cl are more difficult, if not impossible, for the animals to distinguish. The results of the prior discrimination experiment (Geran et al., 2002) confirmed these predictions and suggested that the amiloride-insensitive fibers of the facial nerve were necessary for discriminations involving NH_4Cl, but the sufficiency of this input was not ascertained.

Functional sufficiency of facial nerve input was tested in the current experiment by transecting the glossopharyngeal (GL) nerve. This is not an absolute test of sufficiency as the superior laryngeal branch (SLN) of the vagus nerve is still intact. However, this nerve is thought to be more important for airway protection than taste quality perception (see Dickman & Smith, 1988; St. John & Spector, 1998; Smith & Hanamori, 1991). The GL innervates about 60% of the taste buds in the oral cavity of the rat and contains fibers narrowly-tuned for salts and acids as well as fibers that are highly responsive to compounds described as “bitter” by human subjects (Frank, 1991). Humans have also reported that NH_4Cl and KCl contain both “bitter” and “salty” components (van der Klaauw & Smith, 1995), making it possible that 1 or more types of narrowly-tuned fibers in the GL of the rat are activated by NH_4Cl and/or KCl. It is also possible that discrimination could depend upon GL input due to the large number of taste buds innervated by this nerve. To date, GL transection has not produced substantial decrements in performance on discrimination or recognition tasks involving taste quality. These tasks include sucrose vs. maltose, citric acid vs. quinine, quinine vs. KCl and NaCl vs. KCl (see St. John, Markison, Guagliardo et al., 1997; St. John & Spector, 1997; St. John & Spector, 1998; Spector & Grill, 1992; Spector et al., 1997). Instead, performance

on these tasks has been significantly affected by transection of one or both of the gustatory branches of the facial nerve (see St. John & Spector, 1998), in spite of the fact that the CT and GSP together innervate only about half the number of taste buds innervated by the GL (Miller, 1995). The remaining 5-10% of taste buds are innervated by the SLN (Travers & Nicklas, 1990).

Methods

Subjects

A total of 32 adult male Sprague-Dawley rats (Charles River Breeders, Wilmington, MA) were used in this experiment. These rats were tested in 2 groups of 16. Each animal weighed between 250 and 300g at the start of training and was given *ad libitum* access to PMI 5001 pellets (PMI Nutrition International, Brentwood, MO) at all times while in the home cage. Access to distilled water was restricted to encourage performance during testing. Water bottles were removed from home cages approximately 24 hours prior to training (or testing) every Monday and replaced every Friday following the last session. Rats were able to gain access to water Monday through Friday by pressing the correct lever while in the apparatus. During the week, animals were closely monitored for excessive dehydration. Rats received supplemental water if they appeared dehydrated or if body weight decreased to 85% of the *ad libitum* weight calculated each week. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida.

Apparatus and Trial Structure

Animals received training and testing in an operant chamber modified for taste research. This gustometer apparatus contains one spout for stimulus presentation and one for delivery of the reinforcer. It also contains two levers, one on either side of a spout-

access slot (see Spector, Andrews-Labinski, & Letterio, 1990 for further details). Each animal was allowed to complete as many trials as possible during a 40-min session. Each trial began when the rat made contact with the sample spout twice within 250 ms. This contact completed a low amplitude (< 50 nA) circuit and caused a solenoid valve to open with each subsequent lick so that the stimulus was delivered to the rat's tongue. Each sample phase lasted 5 licks or 3 s whichever came first. This was followed by a 5-s decision phase, during which the stimulus spout rotated out of reach. If the rat pressed the correct lever (i.e., the lever associated with the stimulus during training), it received access to distilled water (20 licks or 10s) via the reinforcement spout. If the rat pressed the incorrect lever or failed to press either lever, the trial ended and the animal received a 20-s time out. An intertrial interval of 10s followed both the reinforcement and time-out phases. At this time, the lights in the chamber were extinguished and the sample spout was rotated over a funnel, rinsed with distilled water and blown dry with pressurized air. Sessions were controlled automatically by a computer and white noise was present throughout each session.

Training Procedure

Rats were counterbalanced for lever and stimulus. Half of the rats (i.e., 16) were trained to discriminate NaCl from NH_4Cl and half were trained to discriminate KCl from NH_4Cl. Training began with shaping the rats to press one lever following presentation of a single stimulus (0.2 M NH_4Cl, 0.2 M KCl or 0.2 M NaCl). Stimuli were made each morning using reagent grade chemicals (Fisher Scientific, Orlando, FL). Once the rat had performed the target behavior for one session without aid, the animal was shaped to press the other lever in response to the other stimulus. This process took approximately 2 weeks. The animals were then switched to the alternation phase during which one

stimulus was presented on each trial until the animal pressed the correct lever on a fixed number of trials. Once the criterion was reached, the other stimulus was presented. It was not necessary for the correct trials to be consecutive. Each rat moved to the next alternation criterion when it performed at 75% or better for the day. The alternation criterion decreased over five days from 8 correct presses to 2. Alternation was followed by discrimination training, during which stimuli were presented in randomized blocks. After two days of 75% performance or better, the limited hold was reduced and the time out increased. These parameters were systematically reduced when the performance of each rat reached the 75% criterion until the last phase of training in which 3 concentrations of each salt were added to the stimulus array (0.4, 0.1, and 0.05 M). Rats were kept on this phase of training until weekly performance was unchanged for 10 sessions (2 weeks). In the current experiment, this last phase of training required an average of 19 sessions. Rats on the KCl discrimination were moved from one phase of training to another simultaneously with rats on the NaCl task. See Table 3-1 or St. John, Markison, Guagliardo et al., 1997 for more detail concerning the training procedure.

Presurgical Discrimination and Amiloride Testing

After training, rats were tested for five days on the 8-stimulus discrimination array (4 concentrations of 2 salts). The following week all stimuli were dissolved in 100 μM amiloride. Amiloride (Sigma Chemical, St. Louis, MO) was made each afternoon prior to testing in a flask wrapped with aluminum foil to minimize reactions with light and allowed to spin overnight. The salt solutions were then made the next morning using amiloride as the solvent instead of distilled water. Amiloride was also used in place of distilled water as the reinforcer during this phase of testing.

Surgery

After amiloride testing, the rats in each discrimination group were divided into 2 groups counterbalanced for body weight, mean number of trials initiated during discrimination testing and overall proportion correct. Half of the animals in each discrimination (n = 8) received bilateral GL transection (GLX) and the other half received sham transection (SHAM). All rats were anesthetized with an intramuscular injection of ketamine hydrochloride (125 mg/kg body weight) mixed with xylazine hydrochloride (5 mg/kg). An incision was made down the midline of the ventral neck and the musculature and salivary glands retracted until the GL could be blunt-dissected from the hypoglossal and vagus nerves with glass rods. The GL nerve was stretched with curved glass rods and cut with microscissors where it met the hypoglossal and vagus nerves such that approximately 5-10 mm of the nerve was removed. Rats in the sham-transected group received only midline incision followed by retraction of the musculature and salivary glands until the GL was exposed. A small amount of sterile saline was introduced into the wound of each animal before it was closed with nylon sutures. Animals received subcutaneous injections of penicillin (30,000 units Flocillin) and analgesic (2 mg/kg Ketorolac) immediately after surgery and for the next 3 days. Animals were allowed 9 (SHAM) or 10 (GLX) days to recover from surgery. One rat in each of the two GLX groups (KCl vs. NH_4Cl and NaCl vs. NH_4Cl) died the night of surgery. It is suspected that the vagal nerve of these animals was accidentally damaged during surgery.

Postsurgical Testing and the Water Control Test

After the recovery period, animals were tested on the original discrimination for 5 sessions followed by amiloride testing for an additional 5 sessions. These tests were performed exactly as they were prior to surgery (see Table 3-2 for experiment schedule).

Rats in the second testing group (n = 15) received a 1-day water control test upon the completion of amiloride testing. Each fluid reservoir was filled with distilled water with half of these assigned to the left lever and half to the right lever. Thus, any performance significantly better than chance could be attributed to an extraneous cue, such as noise or temperature associated with a particular reservoir.

Histology

Upon completion of the water control test, rats were deeply anesthetized with sodium pentobarbital and transcardially perfused with physiological saline and 10% buffered formalin. The oral tissue of each animal was removed and stored in formalin until it could be analyzed. At this time, the tongue was allowed to soak in distilled water followed by dissection of the circumvallate papilla from both the anterior tongue and the underlying connective tissue. The papilla was embedded in paraffin. It was then sliced into 10 μm sections using a microtome, placed on slides, stained using hematoxylin and eosin and coverslipped. Taste buds were counted under a light microscope by an observer blind to surgical group. The presence of a taste pore or the characteristic fusiform cells in the absence of a taste pore was taken as indication of an intact taste bud. Previous research has shown that taste buds degenerate in the absence of afferent innervation, allowing the use of this assay in testing the completeness of nerve transections (Cheal & Oakley, 1977; Hard af Segerstad et al., 1989; St. John et al., 1995).

Data Analysis

Overall discrimination performance for each rat was based on the number of trials with a correct response divided by the total number of trials with a press for each phase of the experiment collapsed across salt and concentration. Two-way analyses of variance (ANOVAs) were also performed to determine the effects of condition and concentration

on performance to each salt. In cases where the ANOVA revealed a main effect of condition, paired t-tests were performed to determine concentrations for which performance was significantly altered. Paired t-tests were also used to compare overall proportion correct between conditions and independent t-tests were performed in some cases to determine whether between-group differences were significant. Finally, the normal approximation of the binomial distribution was applied to the results of the water control test to determine whether performance was significantly different from chance (Brown & Hollander, 1977). A more conservative Bonferroni procedure was applied to these results in order to adjust for the number of t-tests performed. Statistical significance was set at $p < 0.05$ for all tests.

Results

Presurgical Discrimination Testing

Overall mean presurgical discrimination performance was greater than 90% regardless of stimuli. Furthermore, the performance of rats in the KCl vs. NH_4Cl discrimination group did not differ from that of rats in the NaCl vs. NH_4Cl group ($p > .08$). When the salts were dissolved in 100 μM amiloride, KCl vs. NH_4Cl discrimination remained unchanged, while NaCl vs. NH_4Cl discrimination decreased significantly ($p > .62$ and $p < .001$ respectively. Figures 3-1 and 3-2). Two-way ANOVAs (condition x concentration) for each salt in the latter condition indicated an effect of condition for both NaCl and NH_4Cl ($F(1,15) > 47$, $p < .001$ for both). Furthermore, paired t-tests indicated that performance on each concentration of each salt (NaCl and NH_4Cl) declined significantly with amiloride treatment ($p < .004$ for each. Figure 3-3).

Postsurgical Testing and Histology

Mean KCl vs. NH_4Cl performance was significantly lower ($p < .03$, paired t-test) for GLX rats after surgery than before surgery (post-GLX mean = 88% $\pm$ 2 vs. pre-GLX mean = 92% $\pm$ 2. Figure 3-4.). Performance of sham-transected rats also dropped with surgery but was not significant. Both presurgical and postsurgical performance values for the GLX group, however, failed to differ from the values obtained for rats in the SHAM group for the same condition ($p > .88$ for both). Two-way (condition x concentration) ANOVAs for the pre- vs. post-GLX performance of each salt indicated a condition effect for NH_4Cl, but not KCl ($F(1,6) = 6.5$, $p < .05$ and $F(1,6) = 5.4$, $p > .05$, respectively). A post-hoc analysis indicated a significant change in performance with transection for 0.2 M NH_4Cl only ($p < .04$).

Mean NaCl vs. NH_4Cl performance declined significantly following sham-surgery but not GL transection ($p < .03$ vs. $p > .07$, respectively. Figure 3-5.). Two-way (condition x concentration) ANOVAs, however, failed to indicate significant effects of condition or the interaction of condition and concentration for either salt in sham-transected rats ($F(1,6) < 5.7$, $p > .05$ for both salts and $F(3, 18) < .5$, $p > 68$ for both interactions. Figure 3-6). In addition, amiloride treatment did not significantly affect the postsurgical performance of GLX rats trained to discriminate KCl from NH_4Cl (Figure 3-7). Nor did GL transection significantly impair NaCl vs. NH_4Cl discrimination in the presence of amiloride (Figures 3-8 & 3-9).

One rat performed significantly better than chance (i.e., 50%) on the water control test (60% performance, $p < .03$ using a one-tailed test). After performing a Bonferroni adjustment, this performance was no longer significant ($z = 1.99$, $p > .34$. Figure 3-10).

Rats in the GLX group had significantly fewer ($p < .001$) circumvallate taste buds than rats in the SHAM group (0.14 ± 0.14 and 422 ± 19 respectively).

Discussion

Presurgical Discrimination Testing

This study replicated the results of our previous NH_4Cl discrimination experiment (Geran et al., 2002) in that rats on both the KCl vs. NH_4Cl and the NaCl vs. NH_4Cl tasks were clearly able to discriminate between the 2 salts. The prior study was also replicated with regard to the effect of amiloride on these discriminations. Sodium chloride vs. NH_4Cl performance was compromised (11% decline in mean performance, $p < .001$) by the addition of 100 μM amiloride, while KCl vs. NH_4Cl was not significantly affected. Additionally, this impairment was observed at each concentration of both NaCl and NH_4Cl, and performance remained above chance at each concentration (Figure 3-3). This concentration-response pattern is unlike the more one-sided impairment in NaCl vs. KCl discrimination observed with amiloride and less substantial (Spector et al., 1996). In the NaCl vs. KCl task, performance on NaCl trials dropped below chance with amiloride while performance on KCl trials was not significantly altered. Thus, the rats pressed the lever associated with KCl on NaCl trials in the presence of amiloride, suggesting that the taste qualities of KCl and NaCl + amiloride are very similar. This conclusion is supported by additional data from conditioned taste aversion tests and sham-intake experiments in sodium-depleted rats (Hill et al., 1990; Roitman & Bernstein, 1999).

The prior NH_4Cl discrimination experiment indicated similar concentration-response patterns for both the KCl vs. NH_4Cl task and the NaCl vs. NH_4Cl tasks with amiloride (Geran et al., 2002). Although performance to each concentration was well above chance for both the previous and current experiments, in general there was a slight nonsignificant

decline in performance at the 0.05 M NH_4Cl concentration when tested against NaCl + amiloride. This decline mirrors that observed in the KCl vs. NH_4Cl discrimination group both in the presence and absence of amiloride (Figure 3-3; Geran et al., 2002). The fact that this decline did not result in near-chance performance and that the concentrations chosen span almost an order of magnitude suggest that the animals were most likely discriminating based on taste quality rather than intensity, although this has not been tested directly. Detection threshold tasks have indicated that all 3 stimuli (NaCl dissolved in amiloride, KCl and NH_4Cl) are detected by rats at concentrations lower than those tested in the discrimination task (Geran et al., 1999; Geran & Spector, 2000a; Chapter 4). Although the concentrations tested are detectable to the rat, it is not clear how the stimuli compare in terms of suprathreshold intensity.

Postsurgical Discrimination Testing

Potassium chloride vs. NH_4Cl discrimination performance declined slightly but significantly ($p < .03$) following GL transection, while sham transection did not significantly affect performance (Figure 3-4). Interestingly, the decline in performance with surgery was very similar for both GL and sham-transected rats (4% vs. 3%, respectively) and 2-group t-tests failed to indicate significance for either pre- or postsurgical performance. This suggests that perhaps the small number of animals in this group contributed to significance and that the data might simply have been less variable in the GLX group. In support of this interpretation, the difference in standard deviation for the SHAM rats (.065) is almost twice that of the GLX rats (.035). Regardless of statistical significance, a decline in performance from 92% to 88% is meager at best, particularly when compared to a decline from 90% or better to approximately 50% with combined transection of the CT and GSP nerves (Geran et al., 2002).

The performance of rats in the NaCl vs. NH_4Cl discrimination group was not affected by GL transection, but was affected by sham-transection ($p < .03$). Again, this appears to be the result of a small number of subjects combined with low variability (Figure 3-5). Together, these findings suggest that the GL is not necessary for rats to discriminate between NH_4Cl and either NaCl or KCl. In order to focus on the sufficiency of amiloride-insensitive units within the facial nerve, the rats were tested again after surgery in the presence of amiloride. As expected, this drug had no discernable effect on the postsurgical KCl vs. NH_4Cl performance of GL-transected rats (Figure 3-7) and the combination of GLX and amiloride did not impair NaCl vs. NH_4Cl performance beyond that seen with amiloride alone (Figure 3-8). Thus, every rat tested remained able to perform at presurgical levels even after removal of essentially all gustatory input but that of the amiloride-insensitive fibers of the facial nerve.

Every rat except 1 failed to perform better than chance on the water control test, suggesting that these animals were under stimulus control. This rat scored only 60% on the water control test but consistently performed at over 95%, even after GLX, on all other phases of the experiment. This suggests that significance on the water control test might have been due more to the number of t-tests performed than to the perception of an extraneous cue. This interpretation is supported by the Bonferroni adjustment.

Potential Mechanisms Underlying KCl vs. NH_4Cl Discrimination

Although, the animals in this experiment were clearly able to discriminate between KCl and NH_4Cl, the basis for this discrimination is not obvious. One possibility is that a population of taste receptor cells innervated by the facial nerve exists that contains ion channels selectively permeable to either K^+ or NH_4^+. Recent whole cell recordings have shown that although the vast majority of TRCs responsive to 0.1 M KCl or 0.1 M NH_4Cl

are responsive to both stimuli, a few cells respond to only one of these stimuli (Gilbertson et al., 2001). Thus, although unlikely, there is some evidence that such a mechanism might lead to KCl vs. NH_4Cl discrimination in the rat. It is also possible that a general cation receptor, like that described by DeSimone and colleagues (2001), might exhibit different kinetics for K^+ and NH_4^+ that could result in discrimination. Nishijo and Norgren (1997) reported that a group of neurons in the parabrachial nucleus (PBN) was highly responsive to NH_4Cl, but not KCl. Most of the neurons in the PBN that responded to NH_4Cl, however, also responded to KCl. It is not known whether the selectivity of TRC or PBN responses to NH_4Cl changes with stimulus concentration. Regardless of the basis for KCl vs. NH_4Cl discrimination, it is clear that this discrimination exists and depends upon input from the gustatory branches of the facial nerve. The implications of this experiment in regard to taste coding are given further consideration in the final chapter.

Summary

These data support the contention that the facial nerve is more important than the GL nerve for tasks involving taste quality (see St. John & Spector, 1998) in spite of the fact that the GL innervates roughly twice the number of taste buds (Miller, 1995). The possibility remains that vagal, olfactory or somatosensory afferents may provide input that could be useful in KCl or NaCl vs. NH_4Cl performance, making the input from the amiloride-insensitive units of the facial nerve necessary but not entirely sufficient for one or both of these tasks. At present, there is no evidence of such input affecting the perception of salt taste.

Table 3-1. Training Schedule

# of Days	Phase	Time Out (s)	Limited Hold[a] (s)	Stimuli	Stimulus Presentation Schedule
6	Shaping I	none	180	0.2 M NH_4Cl, KCl **or** NaCl	constant
3	Shaping II [b]	none	180	Same as above	constant
8	Alternation [c]	10	15	0.2 M NH_4Cl **and** either KCl or NaCl	alternated after "x" correct responses
2	Discrimination Training I	10	10	Same as above	semi-random [d]
3	Discrimination Training II	20	10	Same as above	semi-random
19	Discrimination Training III	20	5	0.05, 0.1, 0.2, & 0.4 M NH_4Cl and either KCl or NaCl	semi-random

[a] Limited hold refers to the amount of time the rat is given to make a response.
[b] During Shaping II the rat is trained on the opposite stimulus and lever as in Shaping I.
[c] A stimulus is presented repeatedly until a certain number of correct responses are made. This required number of responses, known as the alternation criterion, decreases with each session. Eight for the first session, six for the second, four for the third, three for the fourth, and two for the fifth. It is not necessary that the correct responses be consecutive.
[d] Stimuli were presented in randomized blocks.

Table 3-2. Experiment Schedule.

Phase	**# of Sessions (or Days)**
Training	41
Presurgical Discrimination Testing	5
Presurgical Amiloride Testing	5
Surgery	2
Recovery	9-10
Postsurgical Discrimination Testing	5
Postsurgical Amiloride Testing	5
Water Control Test	1

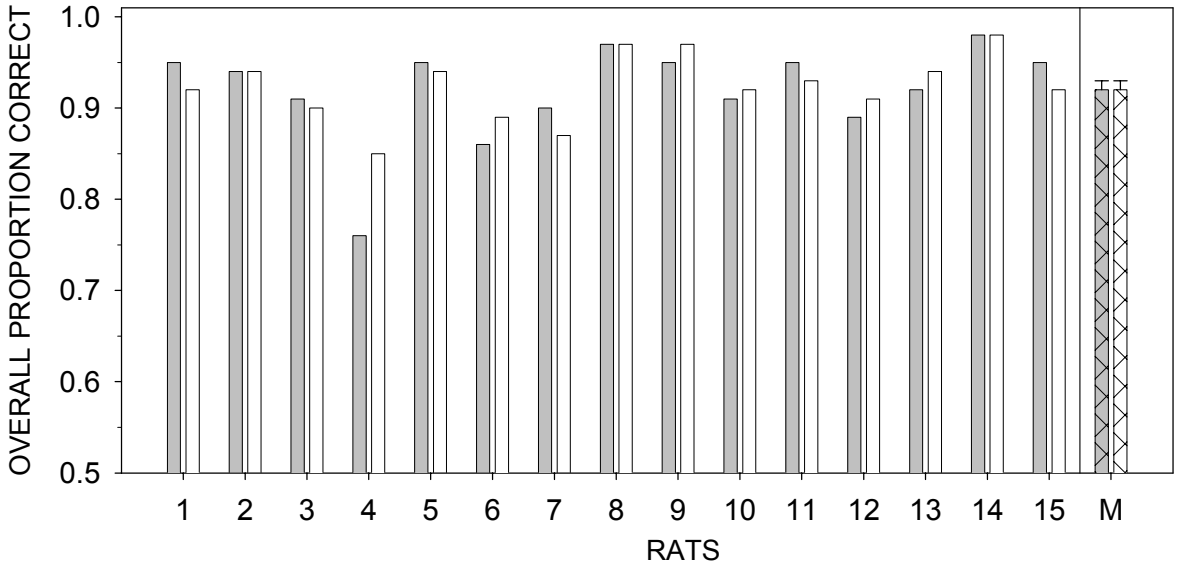

Figure 3-1. Presurgical KCl vs. NH_4Cl discrimination with and without amiloride. Overall proportion correct for each individual with (white) and without (gray) 100 μM amiloride and followed by the mean (M). Note that 50% correct performance (chance) is used as the origin of the y-axis. Amiloride did not significantly impair discrimination performance.

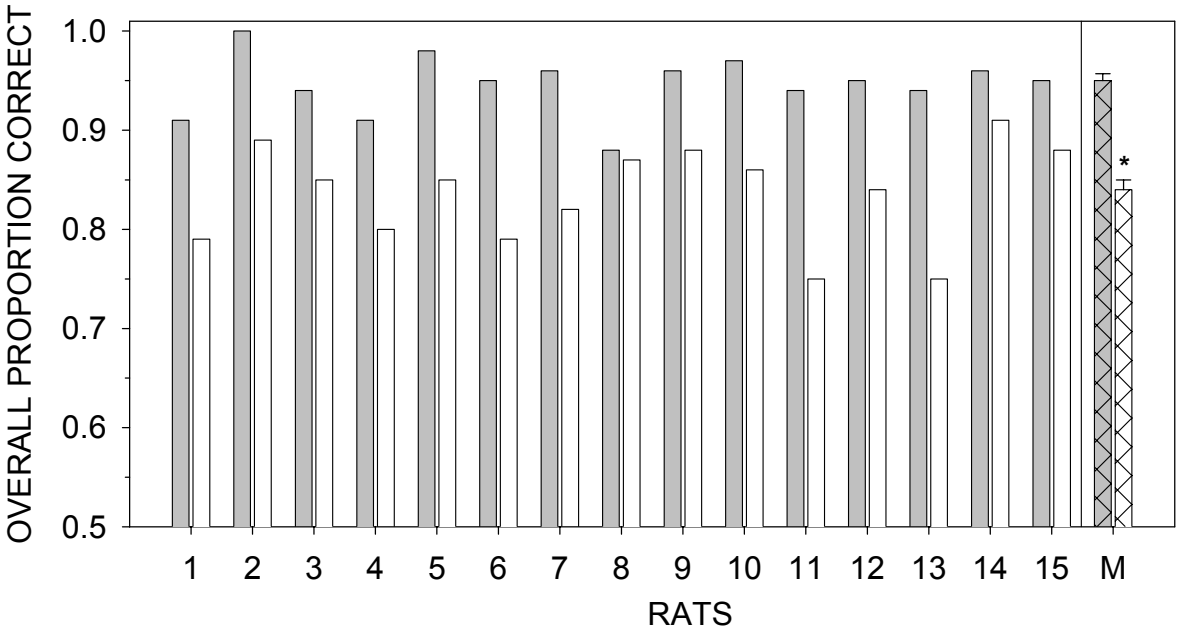

Figure 3-2. Presurgical NaCl vs. NH_4Cl discrimination with and without amiloride. Overall proportion correct for each individual with (white) and without (gray) 100 μM amiloride and followed by the mean (M). Note that 50% correct performance (chance) is used as the origin of the y-axis. Amiloride significantly impaired mean performance ($p < .001$).

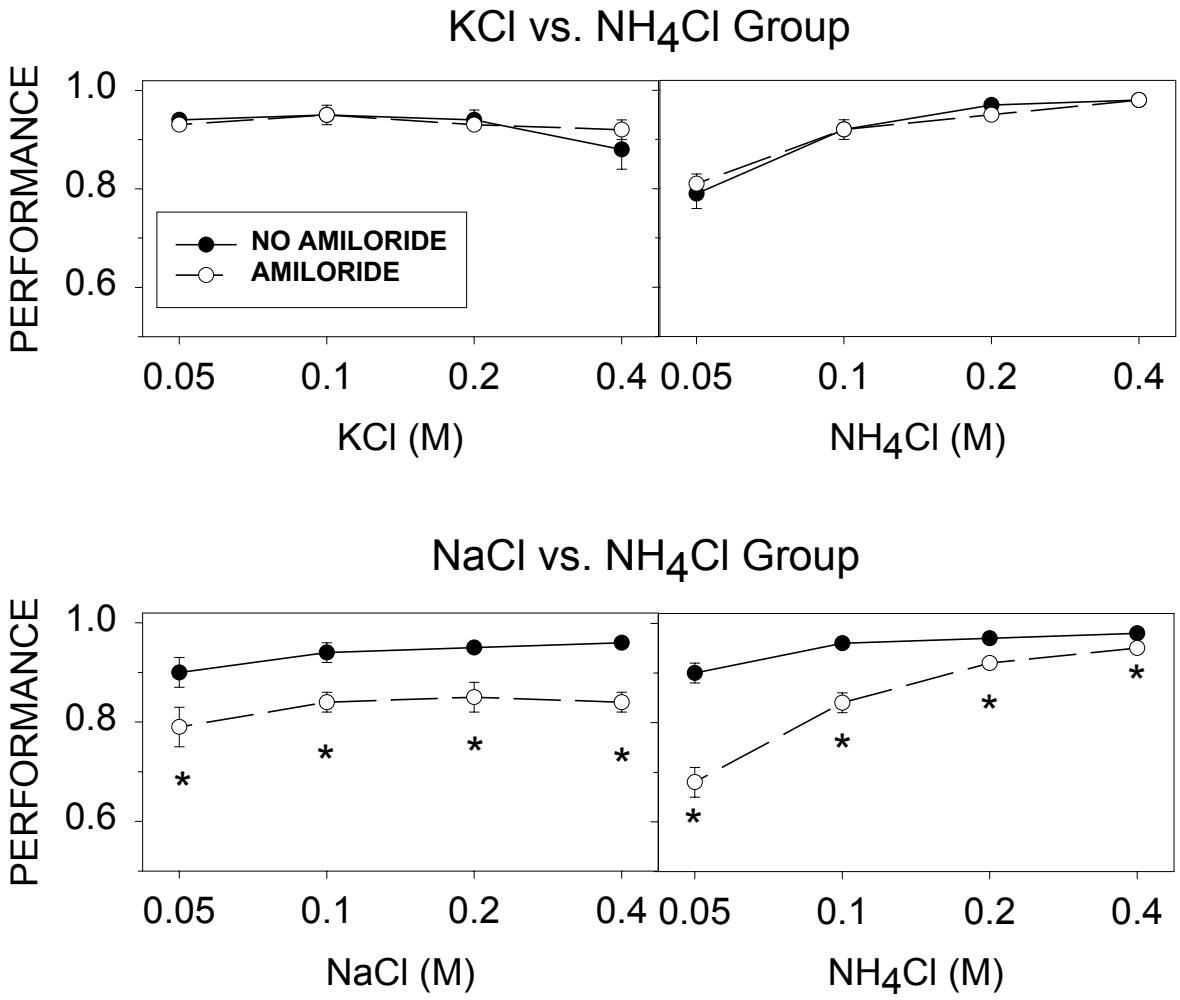

Figure 3-3. Mean presurgical performance by concentration. Performance is separated according to task (KCl vs. NH_4Cl: top, NaCl vs. NH_4Cl: bottom) and salt (NaCl or KCl: left, NH_4Cl: right). Asterisks indicate significant differences between discrimination testing in the presence (white) and absence (black) of 100 μM amiloride ($p < .004$ for each). Note that 50% performance (chance) is used as the origin of each y-axis.

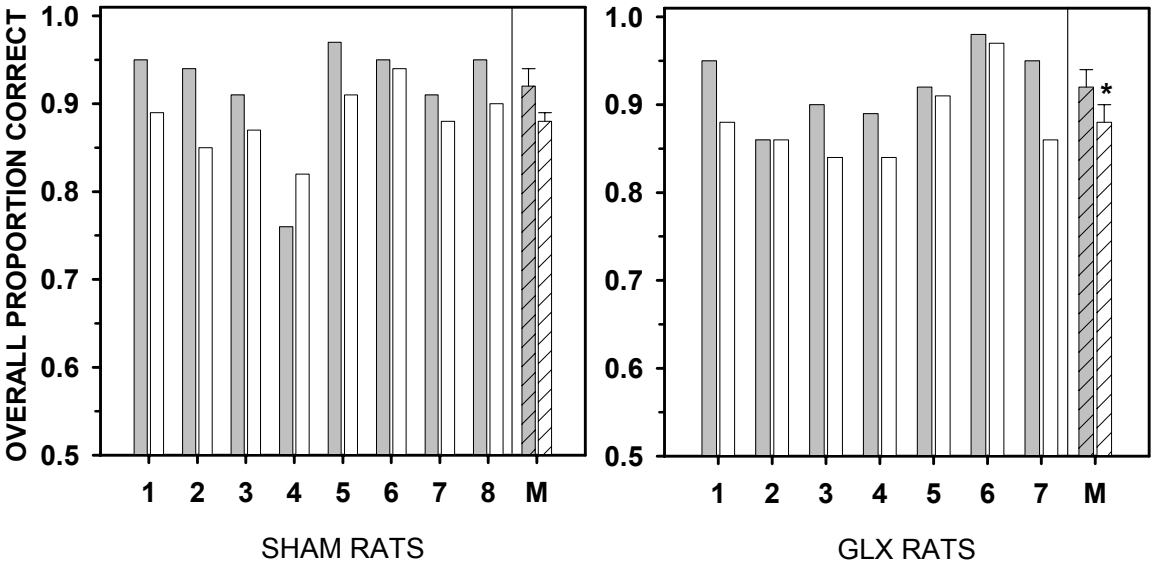

Figure 3-4. Pre- vs. postsurgical performance on the KCl vs. NH_4Cl task. Rats that underwent bilateral glossopharyngeal nerve transection (GLX) are shown on the right and sham-transected (SHAM) rats on the left. Each graph contains presurgical (gray) and postsurgical (white) performance for each rat followed by the mean (M) for each surgical group. Discrimination performance dropped slightly with transection ($p < .03$), but performance of GLX rats was not significantly different from sham-operated controls either before or after surgery ($p > .88$ for both).

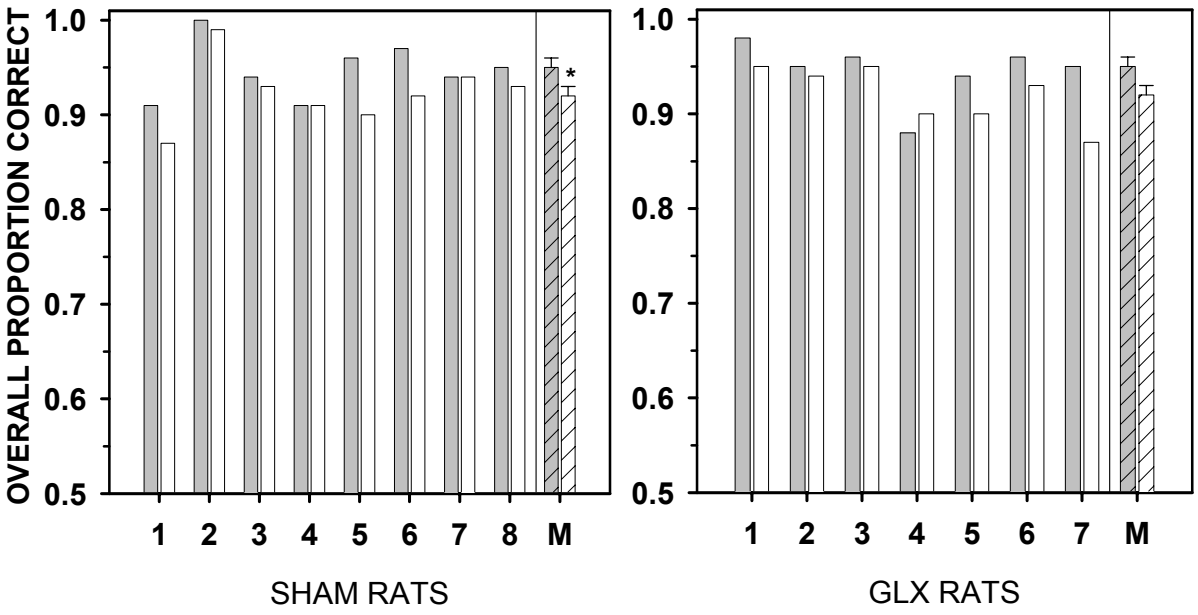

Figure 3-5. Pre- vs. postsurgical performance on the NaCl vs. NH_4Cl task. Rats that underwent bilateral glossopharyngeal nerve transection (GLX) are shown on the right and sham-transected (SHAM) rats on the left. Each graph contains presurgical (gray) and postsurgical (white) performance for each rat followed by the mean (M) for each surgical group. Discrimination performance dropped slightly following sham transection only ($p < .03$).

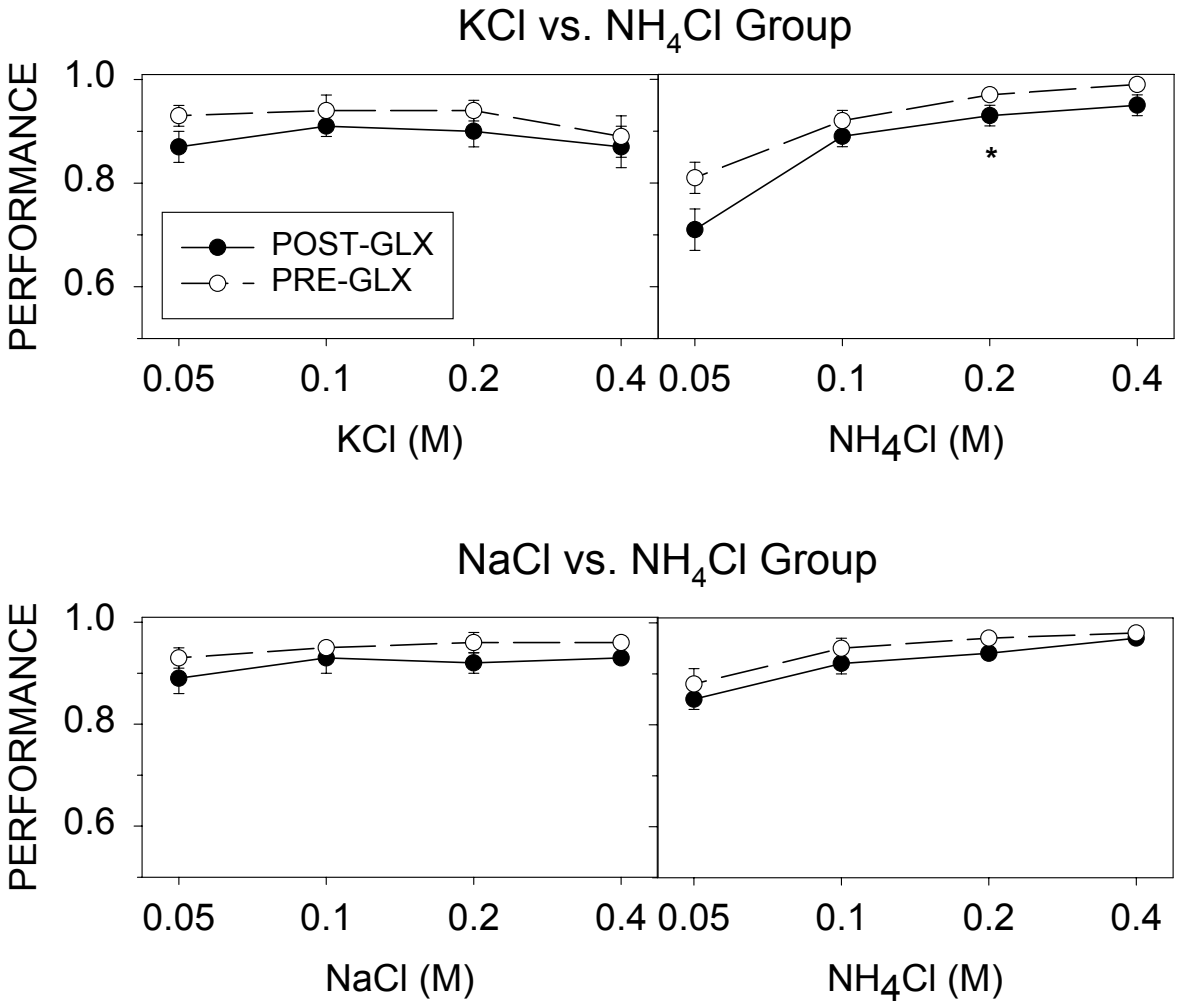

Figure 3-6. Mean presurgical vs. postsurgical performance by concentration. All data points are from rats in the GL transection group. Performance is separated according to task (KCl vs. NH_4Cl: top, NaCl vs. NH_4Cl: bottom) and salt (NaCl or KCl: left, NH_4Cl: right). Asterisks indicate significant differences between presurgical (white) and postsurgical (black) discrimination ($p < .04$). Note that 50% performance (chance) is used as the origin of each y-axis.

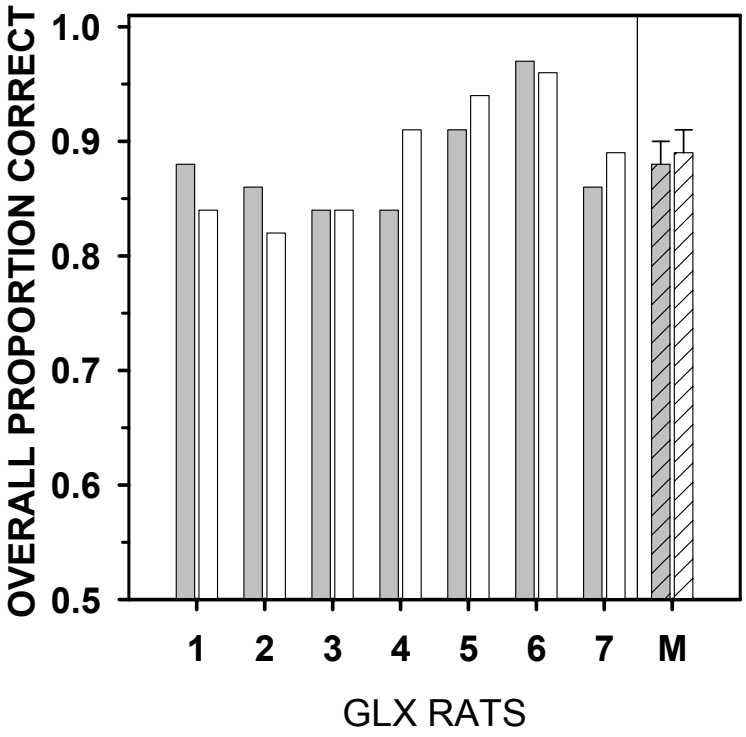

Figure 3-7. Effect of amiloride on postsurgical KCl vs. NH_4Cl discrimination. All data are from GL-transected (GLX) rats. Amiloride (white) did not further impair discrimination performance following surgery (gray).

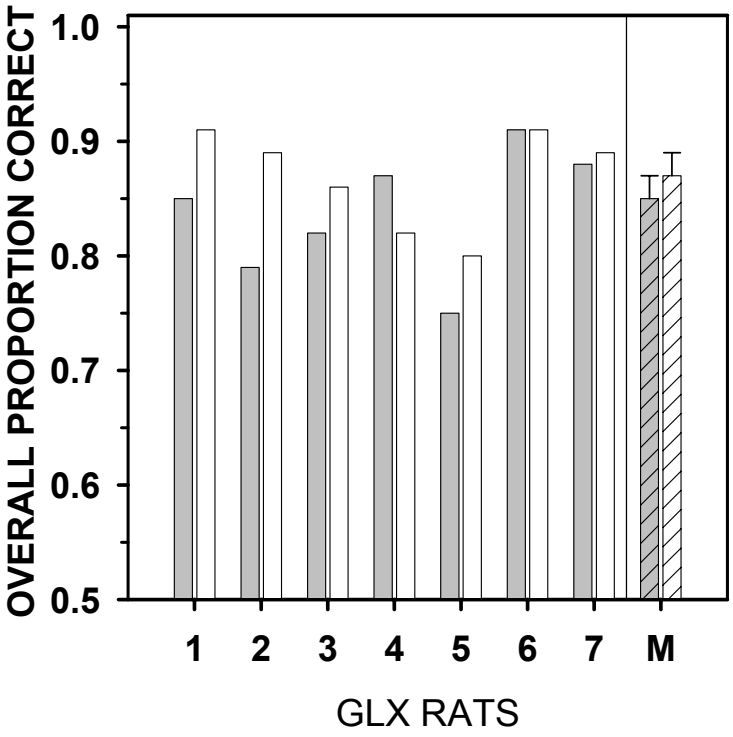

Figure 3-8. Effect of glossopharyngeal transection on NaCl vs. NH_4Cl performance in the presence of amiloride. All data are from rats in the GL transection (GLX) group. Transection (white) did not further impair discrimination performance in the presence of amiloride. Presurgical amiloride performance is shown in gray.

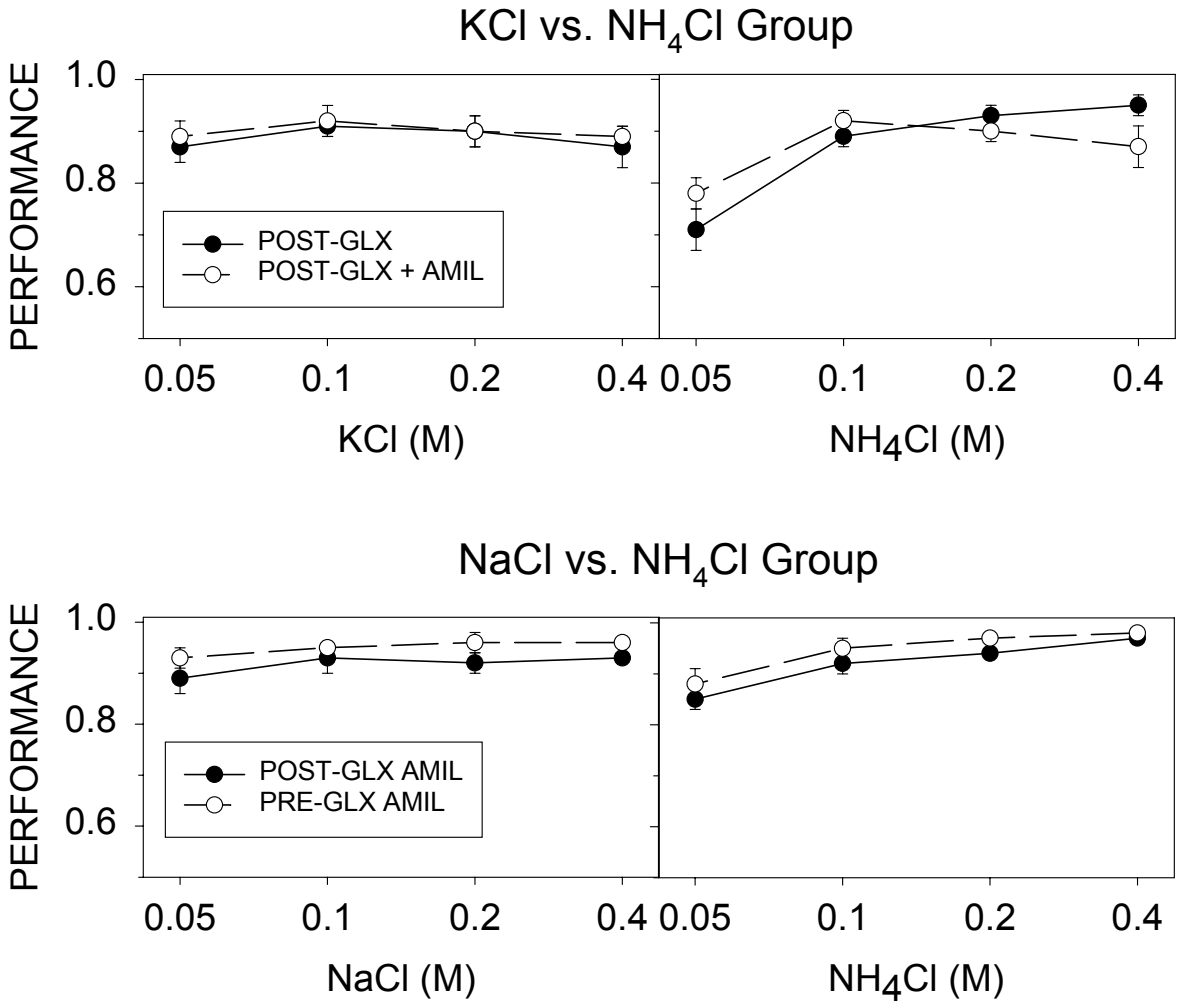

Figure 3-9. Mean comparisons of postsurgical amiloride performance by concentration. All data are from rats in the GL transection group. Performance is separated according to task (KCl vs. NH_4Cl: top, NaCl vs. NH_4Cl: bottom) and salt (NaCl or KCl: left, NH_4Cl: right). In the top graphs, there is no difference between postsurgical performance (black) and postsurgical performance in the presence of amiloride (white). In the bottom graphs, there is no difference between presurgical (white) and postsurgical (black) performance in the presence of amiloride. Note that 50% performance (chance) is used as the origin of each y-axis.

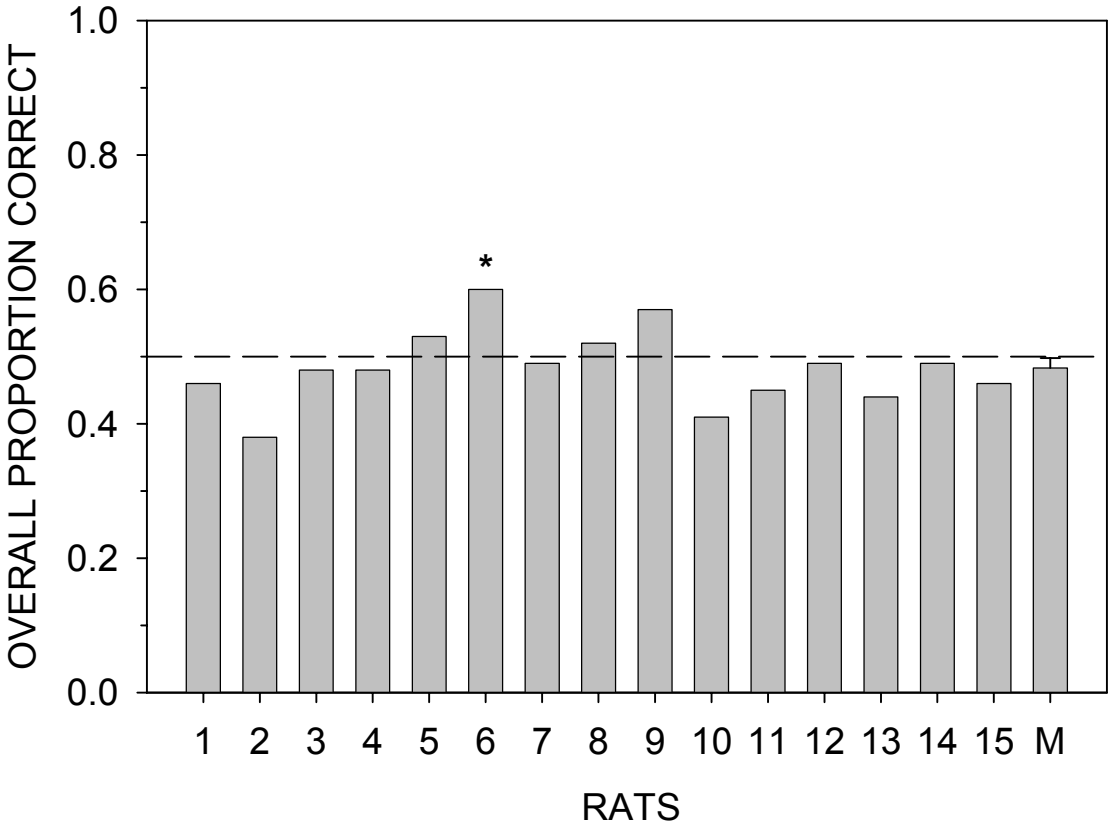

Figure 3-10. Water control test. After testing was complete, all fluid reservoirs were filled with water and assigned to either the left or right lever to assess each rat's ability to respond to extraneous cues. One rat performed significantly better than chance using a one-tailed test ($p < .03$). This significance disappeared when corrected for the number of t-tests performed ($p > .34$). A dashed line represents 50% (chance) performance.

CHAPTER 4
AMILORIDE-INSENSITIVE UNITS OF THE CHORDA TYMPANI NERVE ARE NECESSARY FOR NORMAL AMMONIUM CHLORIDE DETECTABILITY IN THE RAT

Background

The 3 main gustatory nerves of the rat are highly responsive to NH_4Cl at mid to high concentrations (Frank 1991; Frank et al., 1983; Kitada et al, 1998; Nejad, 1986; Sollars & Hill, 1998). For this reason, NH_4Cl is often used as the standard when recording from taste afferents, but very little is known about the perceptual characteristics of this salt. Human subjects have reported that NH_4Cl contains both "salty" and "bitter" components (van der Klaauw & Smith, 1995). Because direct quality scaling and magnitude estimation procedures cannot be used when working with animal subjects, analyses of the perceived taste quality of NH_4Cl in rodents have used conditioned shock avoidance and conditioned taste aversion to measure generalization to salts and other stimuli. All 3 of the published studies using rats have concluded that a conditioned avoidance response or aversion to NH_4Cl generalizes strongly to KCl, and weakly to NaCl (Erickson, 1963; Hill et al., 1990; Morrison, 1967). These results have been interpreted as evidence that NH_4Cl shares a common taste quality with KCl, but not NaCl. This supports the theory that NH_4^+ and K^+ activate at least one common transduction mechanism in the oral cavity (DeSimone et al., 2001; Kloub et al., 1997), while a separate transduction pathway exists for Na^+ in the rat (Brand et al., 1985; DeSimone & Ferrell, 1985; Ninomiya & Funakoshi, 1988). Additional transduction sites might also exist that are more specific for either

NH_4^+ or K^+ as rats have been shown to discriminate easily between KCl and NH_4Cl in spite of evident similarities in taste quality (Geran et al., 2002).

There is some controversy as to whether the epithelial sodium channel (ENaC) blocker amiloride inhibits only sodium and lithium responses in the CT and GSP nerves, or whether its action is more general, reducing neural responding to potassium and ammonium salts as well (see Lundy & Contreras, 1999; Minear et al., 1996). Researchers have demonstrated maximal whole-nerve CT inhibition of up to 48% at .05 M NH_4Cl with the addition of 100 μM amiloride (Kloub et al., 1997. See also Lundy & Contreras, 1997; Lundy et al., 1997). Inhibition was less pronounced at higher NH_4Cl concentrations, losing significance at approximately .3 M (Kloub et al., 1997). The magnitude of this suppression (48%) is greater than that observed for the same concentration of NaCl (30% at .05 M) with 100 μM amiloride, but much less than the 70-80% maximal inhibition observed at higher NaCl concentrations (Brand et al., 1985; DeSimone & Ferrell, 1985).

Other researchers, meanwhile, failed to find any appreciable effect of amiloride on CT responding to either KCl or NH_4Cl (Brand et al., 1985; Formaker & Hill, 1988; Hill & Bour, 1985; Hill et al., 1982). These results are supported by the observation that oral amiloride treatment did not inhibit either the potassium or ammonium response of CT and GSP somata in the geniculate ganglion (Lundy & Contreras, 1999) or activity in the subsets of NTS neurons most responsive to NH_4Cl (Giza & Scott, 1991). At the behavioral level, amiloride pretreatment did not affect generalization following acquisition of a conditioned taste aversion to NH_4Cl (Hill et al., 1990). Also, amiloride failed to compromise either KCl detection threshold or NH_4Cl vs. KCl discrimination in

rats (Geran et al., 1999; Geran et al., 2002). Together, these findings suggest that although the effect of amiloride on nonsodium salt responsiveness is perhaps at times statistically significant at the level of the peripheral nervous system, it does not appear to reach significance at higher levels of the gustatory system or lead to behavioral significance.

Taste sensitivity to NH_4Cl was tested in the presence and absence of amiloride to determine whether any inhibition in the periphery might affect the taste-guided behavioral response of the animal. Amiloride suppression of the CT response to NH_4Cl was greatest at low concentrations (Kloub et al., 1997) suggesting that impairment would be most noticeable at the limits of detectability. Detection threshold was also chosen for measurement because amiloride was previously shown to significantly raise the thresholds of both NaCl and Na-gluconate using a similar procedure (Geran & Spector 2000a; 2000b). Earlier detection threshold experiments have also reported that NaCl and KCl sensitivities were compromised by bilateral transection of the CT (CTX) suggesting that input from this nerve is necessary for normal salt detectability (Geran et al, 1999; Kopka & Spector, 2001; Slotnick et al., 1991; Spector, Schwartz & Grill, 1990). To further test this hypothesis NH_4Cl detection threshold was also measured after severing the CT nerves.

Methods

General Methods

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida. Ten adult male Sprague-Dawley rats were placed on a water-restriction schedule with water bottles removed about 24h prior to the start of training or testing on Monday and replaced after the last session Friday. During the

week, animals worked for water access in the gustometer apparatus (see Spector, Andrews-Labinski & Letterio, 1990). Subjects were cared for exactly like subjects in Chapter 3, except where noted. The animals were trained to press one lever immediately after a presentation of 0.2 M NH_4Cl and the other lever immediately after a distilled water presentation. As subjects grew more proficient, more concentrations were added to the stimulus array and the parameters of the trial structure were altered such that the decision phase was reduced and the time out increased. Subjects moved to the next phase of alternation or training when they reached at least a 70% overall performance criterion. See Table 4-1 for the training schedule. Each session lasted 40 min with the rats allowed to complete as many trials as possible during this time. In the last phase of training, stimuli were presented in randomized blocks of 10 consisting of 5 NH_4Cl concentrations and 5 distilled water presentations. This final training phase was exactly like the first week of testing. As performance did not change over the last week of the final training phase (Discrimination Training III), this period was retroactively defined as the first week of presurgical threshold testing.

Testing

Detection threshold was tested over the course of 4 weeks using a total of 8 NH_4Cl concentrations ranging from .00325 to .4 M. Each Monday rats received the standard stimulus array (i.e., 0.4, .2, .1, .05 & .025 M NH_4Cl). A different array was presented Tuesday through Friday containing at least 2 concentrations from the standard array and several lower concentrations. At least one stimulus in the test array was replaced with a lower concentration each week until performance approached a minimum asymptote. Rats were given a second week of testing with the lowest concentration array to increase the number of trials for these stimuli. See Table 4-2 for test stimulus presentation

schedule. During presurgical and postsurgical amiloride testing, 100 μM amiloride was used as the solvent for all NH_4Cl stimuli and in place of water for both stimulus presentations and reinforcement. A water control test was performed following the final postsurgical test to assess whether the rats were capable of responding to extraneous cues unrelated to the chemosensory properties of the NH_4Cl stimuli. This was accomplished by filling each reservoir with distilled water and assigning half of these to the left lever and half to the right. See Table 4-3 for phases of the experiment.

Surgery

Rats were divided into 2 groups counterbalanced for weight, overall performance and number of trials initiated. All rats were anesthetized with a mixture of ketamine (125 mg/kg body weight) and xylazine hydrochloride (5 mg/kg) injected intramuscularly. Five of these rats received bilateral CT nerve cauterization. This was accomplished by retracting the external ear canal to expose the tympanic membrane. The membrane, along with the rim of the ear canal, the malleus and the CT nerve were then cauterized. This procedure stimulates the production of cerumen, which keeps the CT from reinnervating taste buds in the anterior tongue for at least 118 days (Kopka & Spector, 2001). The 5 rats in the sham-transected group had each ear retracted and the tympanic membrane punctured with microforceps. All animals received subcuteneous injections of penicillin (30,000 units Flocillin) and an analgesic (Ketorolac, 2 mg/kg body weight) immediately following surgery and for the next 3 days. Rats were given 6-7 days to recover from surgery before testing resumed.

Histology

After postsurgical testing, rats were deeply anesthetized with sodium pentobarbital (i.p.) and rapidly perfused with saline and 10% buffered formalin. The tongue and palate

were removed and stored in formalin. Staining was accomplished by placing the anterior portion of the tongue from the tip to the intermolar eminence in distilled water for 30 min then dipping it in 0.5% methylene blue until dark and rinsing the tissue with water to remove excess stain. The epithelium of the anterior tongue was then pressed between 2 glass slides and examined under a light microscope. The number of intact fungiform papillae and taste pores were counted on each tongue. Taste pores appeared as small round dots surrounded by blue circles under the microscope (Parks & Whitehead, 1998; St. John et al., 1995). The presence of a discernable blue dot was counted as a pore for the purposes of this experiment. Results using this method correspond with those from a hematoxylin and eosin stain and also correlate with the degeneration and regeneration of taste buds concomitant with denervation and reinnervation by the CT nerve (St John et al., 1995). Histology was performed blind to the rat's surgical treatment.

Data Analysis

The percentage of correct responses on NH_4Cl trials was adjusted for false alarm probability (see Gescheider, 1997). This was accomplished using the following equation for corrected hit rate, or $P(Hit)_c$:

$$P(Hit)_c = \frac{P(Hit) - P(FA)}{1 - P(FA)}$$

where P(Hit) was the proportion of NH_4Cl trials on which the rat pressed the correct lever and P(FA) was the proportion of water trials on which the animal pressed the wrong lever. Hit rates were corrected for each rat at each NH_4Cl concentration. The following logistic function was then used to fit curves to the corrected hit rate values for each animal:

$$f(x) = \frac{a}{1+10^{-b(x-c)}}$$

where a = maximum asymptote of performance, b = slope, x = NH_4Cl $\log_{10}$ concentration and c = threshold. Threshold was defined as the NH_4Cl concentration at one-half the maximum asymptote of performance. Analyses of variance (ANOVAs), paired and independent t-tests and the normal approximation of the binomial distribution (Brown & Hollander, 1977) were used to assess statistical significance. Alpha was set at the conventional .05 level.

Results

Presurgical Detection Threshold

Mean detection threshold for the first 20-session threshold was .012 M NH_4Cl $\pm$ .001. With the addition of 100 μM amiloride, mean threshold decreased significantly ($p < .002$, Figure 4-1). In other words, the rats performed better at near threshold NH_4Cl concentrations with amiloride than prior to treatment. When NH_4Cl threshold was again measured without amiloride, sensitivity improved further still ($p < .006$, mean threshold = .009 M $\pm$ .001. Figure 4-2). Two-way ANOVAs (phase x concentration) of corrected hit rates indicated main effects for both phase: ($F(1,9) > 17$, $p < .003$) and concentration: ($F(7,63) > 468$, $p < .001$) as well as for their interaction ($F(7, 63) > 4$, $p < .002$) when each of the 2 presurgical tests without amiloride was compared to the amiloride test. From the first presurgical measurement to the last, mean threshold decreased by .25 $\log_{10}$ units $\pm$ .03 ($p < .001$; see Figure 4-3 for individual shifts in threshold).

Postsurgical Detection Threshold

Mean NH_4Cl threshold increased following CT transection by .54 $\log_{10}$ units $\pm$ .09 to .04 M ($p < .004$. Figure 4-4). This value differs significantly from that of the sham-operated rats ($p < .001$, see Figure 4-5). Threshold did not change significantly with surgery for sham-operated rats compared with the last presurgical threshold assessment ($p > .08$). Nor did amiloride alter NH_4Cl threshold in either the CT-transected or sham-transected rats after surgery ($p > .29$ for both. Figure 4-6).

Water Control Test and Histology

No rat scored better than chance when all stimuli were replaced with distilled water (one-tailed t-tests using the normal approximation of the binomial distribution, $p > .17$ for each. Figure 4-7). Histological analysis indicated no significant difference in the number of fungiform papillae for the 2 surgical groups (CTX: 106 $\pm$ 34 SHAM: 139 $\pm$ 23. $p > .11$). Rats in the CT-transected group, however, had far fewer taste pores on the anterior tongue than rats in the sham-transected group (6 $\pm$ 8 and 132 $\pm$ 23 respectively, $p < .001$).

Discussion

It is clear from these data that the rats were responding to the chemical properties of the stimuli rather than to extraneous cues such as temperature or sound, as none of the rats scored better than chance on the water control test (Figure 4-7). The lowest mean detection threshold for NH_4Cl (.009 M) recorded in this experiment was slightly higher than that found previously for NaCl (.005 to.006 M) using the same 2-lever operant procedure (Geran & Spector, 2000a; 2000b; Kopka & Spector, 2001). In contrast, the detection threshold for KCl (.04 M) was considerably higher than for either of the other chloride salts tested to date (Geran et al., 1999). The detectability functions for these 3 salts will be compared in greater detail in the final chapter.

Ammonium Chloride Detectability Depends Upon an Amiloride-Insensitive Route of Transduction

A subset of electrophysiological studies has reported that amiloride significantly suppressed CT responding to low and mid-range concentrations of NH_4Cl (Kloub et al., 1997; Lundy & Contreras., 1997; Lundy et al., 1997), suggesting that amiloride might also compromise NH_4Cl detection threshold. Instead, threshold decreased in the presence of the ENaC blocker. Amiloride did not seem to be the cause of the increase in NH_4Cl sensitivity, however, as threshold decreased further still when the rats were tested a second time in the absence of amiloride (Figure 4-2). In addition, amiloride failed to decrease postsurgical threshold for either surgery group. It is unclear whether this decrease in threshold with repeated testing represents an aspect unique to NH_4Cl such as an upregulation of ammonium-sensitive receptor elements. Alternatively, the decrease in threshold could be due to increased performance resulting from previous experience with low concentrations of the stimulus.

Gilbertson and colleagues (1993), reported that vasopressin, a hormone involved in fluid homeostasis, increases the inward Na^+ current through amiloride-sensitive channels in the taste receptor cells of hamsters. Although the amiloride-sensitive transduction pathway of the hamster is different in significant ways from that of rats, it is conceivable that such a mechanism could potentially have been activated by the restricted fluid access experienced by the rats in the current experiment. One would expect such a mechanism to have a greater effect on NaCl threshold than NH_4Cl threshold, however, as this task is more dependent on amiloride-sensitive units. Sodium chloride threshold increases significantly in the presence of amiloride, and returns to the pre-amiloride threshold when tested following amiloride treatment (Geran & Spector, 2000a; 2000b). This suggests that

the decrease in NH_4Cl threshold observed during and after amiloride treatment was not due to an upregulation of amiloride-sensitive receptors, although amiloride-insensitive receptors permeable to NH_4^+ could have been affected. There is currently no evidence to support such a hypothesis, however. In fact, NH_4Cl vs. KCl discrimination performance was unchanged with amiloride treatment (Geran et al., 2002). Thus, the decrease in NH_4Cl threshold with each presurgical test is most likely the result of continued experience with the task.

In addition to having little to no effect on the perceived intensity of NH_4Cl at low concentrations, amiloride also seems to be without effect on the taste quality of this salt. For instance, amiloride treatment failed to compromise performance on a KCl vs. NH_4Cl task (Geran et al., 2002). In addition, taste aversions conditioned to NH_4Cl resulted in the same pattern of generalization regardless of whether the animals were treated with amiloride at the time of conditioning (Hill et al., 1990). Thus, amiloride does not seem to appreciably affect the perceived intensity of NH_4Cl at low concentrations, or its perceived taste quality at mid-range to high concentrations (i.e., .05 to .4 M. Geran et al., 2002; Hill et al., 1990). It is unlikely that the drug compromises NH_4Cl intensity at higher concentrations as the electrophysiology indicates very little suppression, if any, at concentrations above .3 M (Kloub et al., 1997; Lundy et al., 1997). Therefore, it is more likely that amiloride affects the taste quality of NH_4Cl at low concentrations, if it has any effect at all on taste-guided performance to NH_4Cl. The current procedure only measures detection, not recognition (see Gescheider, 1997). Thus, although the rats in this study were able to discriminate NH_4Cl dissolved in amiloride from amiloride alone, they may

have nevertheless been unable to recognize the stimulus as NH_4Cl at near threshold concentrations.

The Chorda Tympani Nerve is Necessary for Normal Ammonium Chloride Detection

Bilateral transection of the CT significantly increased NH_4Cl detection threshold (Figure 4-4), suggesting that the information carried by this nerve is necessary for normal detection of this salt. It is also possible that the GSP and/or GL are necessary, although insufficient, for normal NH_4Cl detection. The result of this experiment, along with the increases previously reported for both KCl and NaCl threshold with CT transection (Geran et al., 1999; Kopka & Spector, 2001; Slotnick et al., 1991; Spector, Schwartz & Grill, 1990), support the hypothesis that the CT nerve is important for normal salt detection. This nerve also appears to be important for the recognition of salt stimuli as CTX impairs performance on a NaCl vs. KCl discrimination task (Kopka et al., 2000; St John, Markison, Guagliardo et al., 1997; Spector & Grill, 1992). It is unclear whether the role of the CT in salt recognition extends to NH_4Cl, as CTX does not alter unconditioned licking to this salt in a 2-bottle preference test (Sollars & Bernstein, 1996). Of course, factors other than taste quality, such as hedonic value or the postingestive consequences of NH_4Cl consumption, could have influenced performance on the 2-bottle test (see Spector, 2000).

Like the CT, the GSP also appears to carry behaviorally-relevant information about salt stimuli (Kopka et al., 2000; Roitman & Bernstein, 1999; St. John, Markison & Spector, 1997). For instance, amiloride abolishes NaCl vs. KCl discrimination while CTX merely impairs it, suggesting that residual discrimination after CTX relies upon amiloride-sensitive, sodium-selective receptors innervated by the GSP (see Kopka et al.,

2000; Roitman & Bernstein, 1999; St. John, Markison & Spector, 1997). The GL is not thought to contain AS taste receptor cells (Doolin & Gilbertson, 1993; Kitada et al., 1998). Thus, both the CT and GSP appear to carry information important for NaCl recognition, and could perhaps also be necessary for normal NH_4Cl recognition. It is not clear at present whether the CT, GSP, or the combined input of the two is required for the rat to accurately perceive the taste quality normally associated with NH_4Cl. If the GSP is required for this task, one would expect CTX to impair NH_4Cl vs. KCl discrimination performance significantly less than combined transection of the CT and GSP.

In contrast, input from the GL does not appear to be necessary for the perception of salt taste quality (Markison et al., 1995; Spector & Grill, 1992; Chapter 3). Instead, the GL appears to be more important for the stimulation of unconditioned aversive gustatory reflexes, like gaping upon contact with quinine, than for the perception of taste quality (St. John & Spector, 1998; Travers et al., 1987). For instance, combined transection of the CT and GSP nerves drops performance on a KCl vs. NH_4Cl discrimination task to chance while GL transection is without effect (Geran et al., 2002; Chapter 3).

Conclusions

Like KCl detection, normal NH_4Cl detectability appears to depend upon amiloride-insensitive receptors innervated by the CT nerve. It is unclear whether the same population of receptors is responsible for both NH_4Cl and KCl detection. The fact that KCl and NH_4Cl are easily discriminated by the rat (Geran et al., 2002) suggests that differences in activation of the NTS exist for the 2 salts but have not yet been found.

The increase in detection threshold for NH_4Cl with CT transection lends further support to the hypothesis that this nerve is necessary for the normal detection of salt stimuli. This manipulation has also impaired both NaCl and KCl detectability in previous

tests (Geran et al., 1999; Kopka & Spector, 2001; Slotnick et al., 1991; Spector, Schwartz & Grill, 1990). Finally, the current experiment was also useful in that it provides a detection threshold for NH_4Cl that can be used to determine testing concentrations for future psychophysical studies.

Table 4-1. Training Schedule

# of Days	Phase	Time Out (s)	Limited Hold[a] (s)	Stimuli	Stimulus Presentation Schedule
9	Shaping I	none	180	0.2 M NH_4Cl **or** DH_2O	constant
4	Shaping II [b]	none	180	0.2 M NH_4Cl or DH_2O	constant
7-14	Alternation [c]	10	15	0.2 M NH_4Cl **and** DH_2O	alternated after "x" correct responses
2	Discrimination Training I	10	10	0.2 M NH_4Cl and DH_2O	semi-random [d]
3	Discrimination Training II	20	10	0.2 M NH_4Cl and DH_2O	semi-random
8-15	Discrimination Training III [e]	20	5	0.025, 0.05, 0.1, 0.2, 0.4 M NH_4Cl and DH_2O	semi-random

[a] Limited hold refers to the amount of time the rat is given to make a response.
[b] During Shaping II the rat is trained on the opposite stimulus and lever as in Shaping I.
[c] A stimulus is presented repeatedly until a certain number of correct responses are made. This required number of responses, known as the alternation criterion, decreases with each session. Eight for the first session, six for the second, four for the third, three for the fourth, and two for the fifth. It is not necessary that the correct responses be consecutive.
[d] Stimuli were presented in randomized blocks.
[e] The number of days shown here includes the first week of presurgical threshold testing.

Table 4-2. Test Stimulus Presentation Schedule

	NH_4Cl Concentrations (M) in Test Array							
	.4	**.2**	**.1**	**.05**	**.025**	**.013**	**.0065**	**.00325**
Week 1 & Mondays: Standard Array	■	■	■	■	■			
Week 2 (Tue to Fri)		■		■	■	■	■	
Week 3 (Tue to Fri)			■	■		■	■	■
Week 4 (Tue to Fri)			■	■		■	■	■

Table 4-3. Experiment Schedule

Phase	**# of Sessions (or Days)**
Training	35
Presurgical Threshold Testing 1	20
Presurgical Amiloride Testing	20
Presurgical Threshold Testing 2	20
Surgery	2
Recovery	6-7
Postsurgical Threshold Testing	20
Postsurgical Amiloride Testing	20
Water Control Test	1

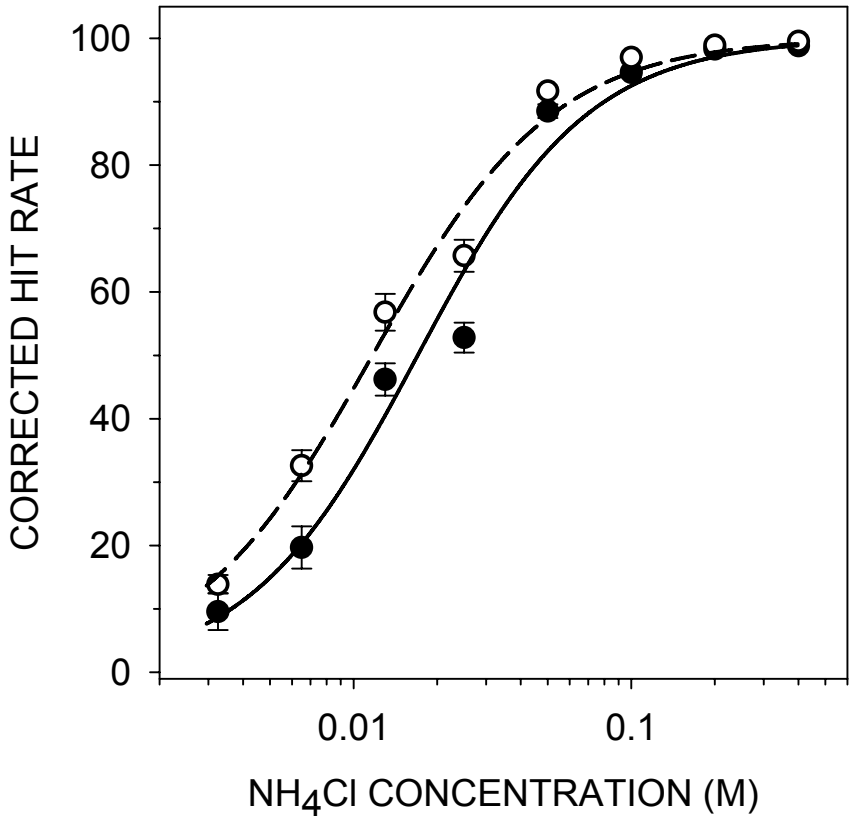

Figure 4-1. Effect of amiloride on NH_4Cl detection. Mean performance with and without amiloride (100 μM). Threshold decreased significantly (i.e. sensitivity increased) with amiloride ($p < .002$). Amiloride performance is represented with open symbols.

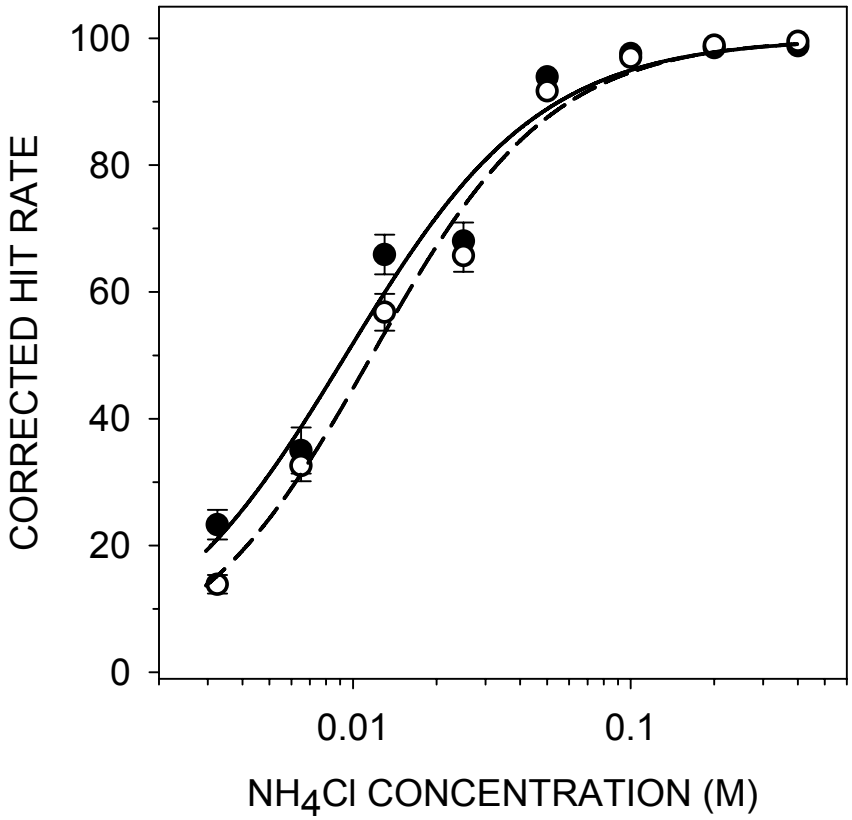

Figure 4-2. NH_4Cl threshold decreased again following amiloride treatment. Threshold decreased significantly following amiloride testing ($p < .006$). Open symbols represent amiloride performance.

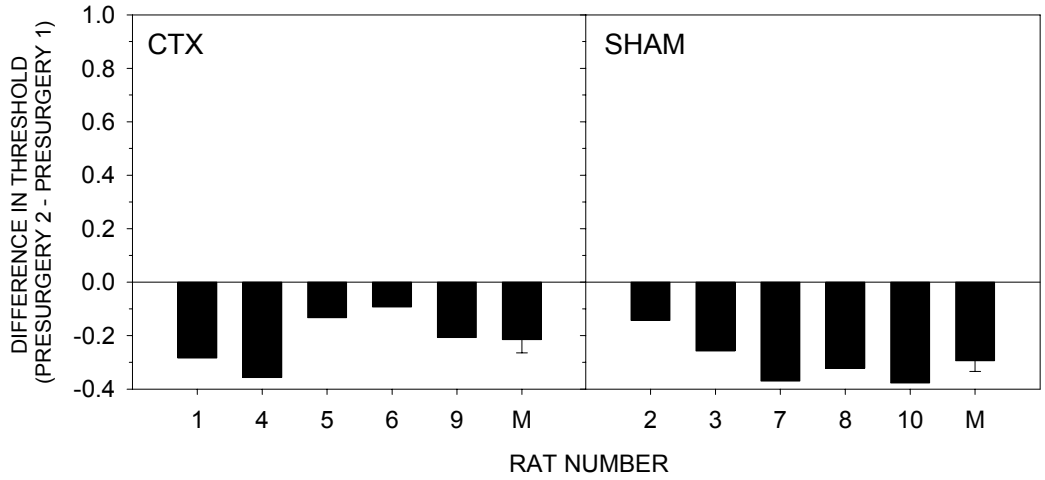

Figure 4-3. Individual shifts in presurgical threshold. Shifts for each animal followed by the mean for each surgical group. All values are before surgery. Each animal showed a decrease in threshold between the first and second measurement of NH_4Cl detectability in the absence of amiloride. Animals on the left underwent transection of the chorda tympani nerve (CT) after these shifts in threshold were measured while animals on the right received sham surgery.

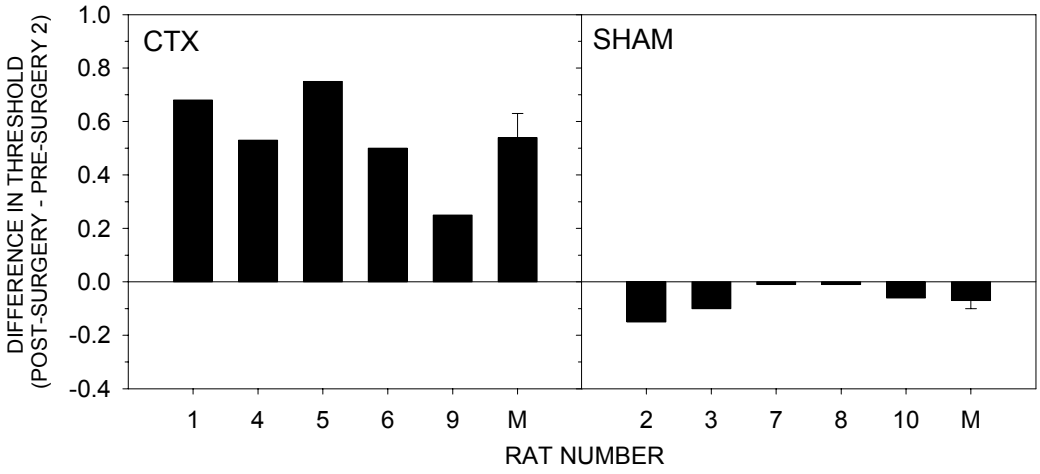

Figure 4-4. Individual shifts in performance with surgery followed by the mean for each group. Animals that received bilateral chorda tympani transection (left) showed a significant increase in threshold ($p < .004$), while sham-operated rats (right) did not.

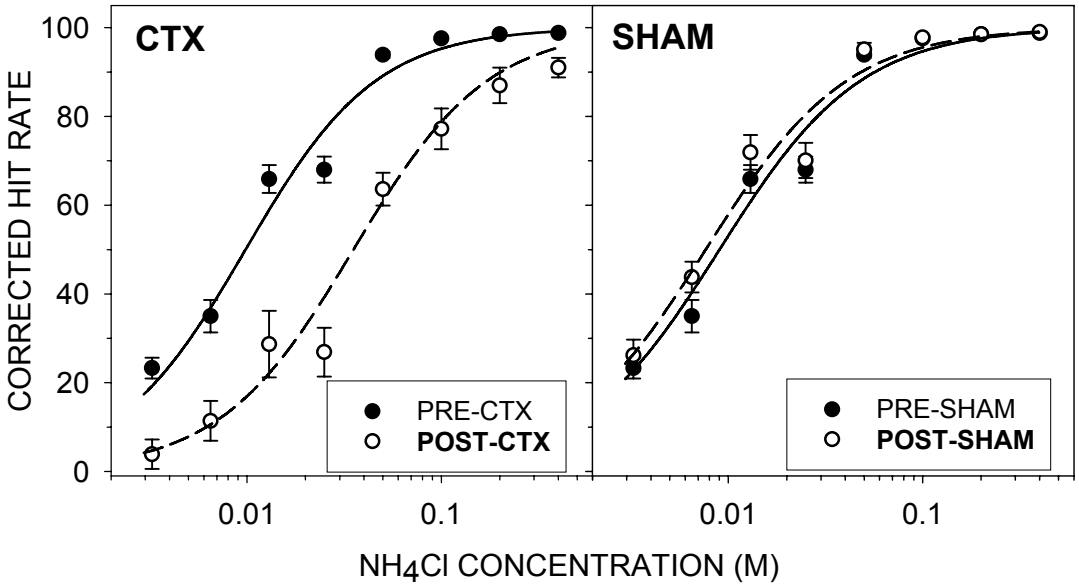

Figure 4-5. NH_4Cl detectability functions pre- and post-surgery. Mean performance for each surgical group. Rats in the chorda tympani transection group (left) had significantly higher thresholds ($p < .004$) following surgery while rats in the sham-operated group (right) did not. Presurgical results are from the second determination of threshold in the absence of amiloride.

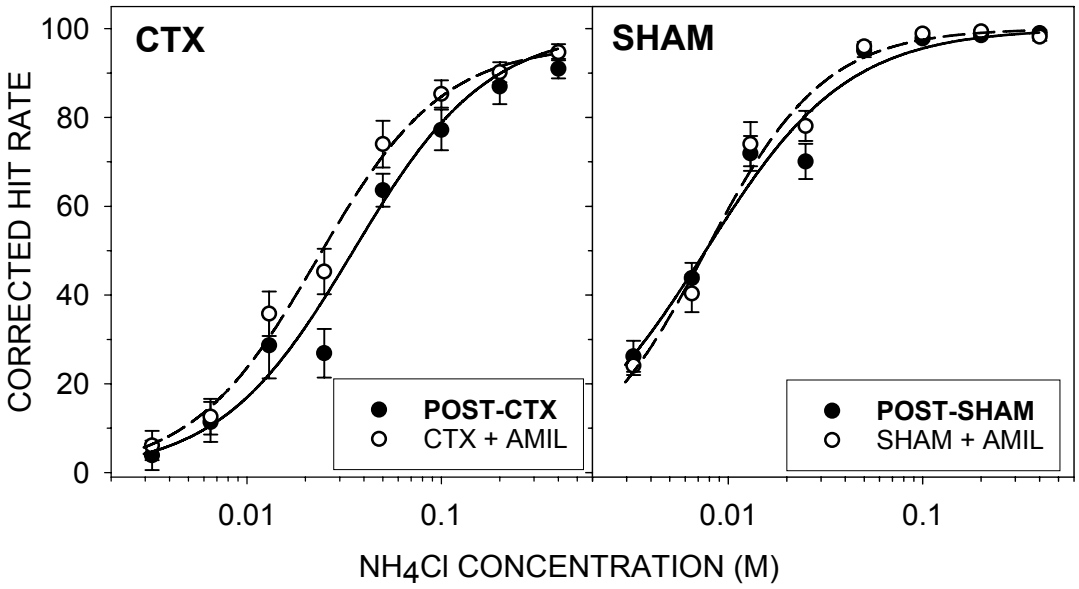

Figure 4-6. Postsurgical NH_4Cl detectability functions with and without amiloride (100 μM). Neither rats in the chorda tympani transected group (left) nor rats in the sham-operated group (right) showed any significant change in NH_4Cl threshold with amiloride.

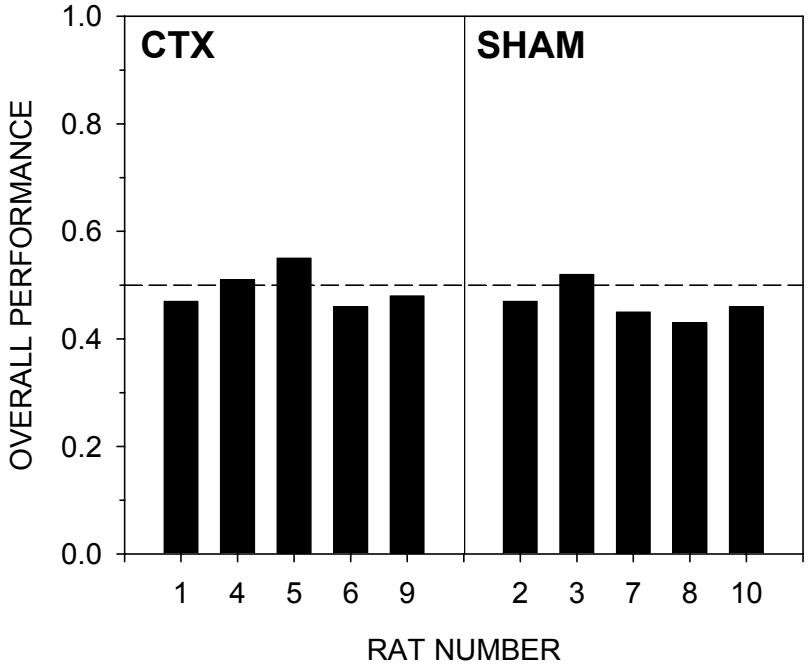

Figure 4-7. Water control test. Individual overall performance when all stimuli were replaced with distilled water. Neither rats in the chorda tympani transected group (left), nor rats in the sham-operated group (right) performed significantly better than chance.

CHAPTER 5
GENERAL DISCUSSION

Discrepancies Between the Electrophysiology and the Behavior In Regard to NH_4Cl

Regardless of whether NH_4Cl vs. KCl discrimination is due to different receptor subtypes or different temporal properties within the same receptor subtype, significant differences in activity at the level of the CNS should be noticeable. At present, no such differences have been observed, although this might be due to the paucity of experiments that include both NH_4Cl and KCl as stimuli. The overall patterns of activity are very similar for NH_4Cl and KCl in the NTS (Erickson, 1963; Nakamura & Norgren, 1993). This similarity in neural responding in conjunction with similar generalization patterns for the two salts in conditioned aversion and avoidance tasks has historically been used to support both the across-fiber pattern theory and the best-stimulus method of classification (see Erickson, 1963; Smith et al., 2000). Although these salts have been shown to taste similar to the rat (Erickson, 1963; Hill et al., 1990; Morrison, 1967), they are also easily discriminated suggesting that coding theorists must also provide an explanation for this phenomenon.

In addition to the small number of CNS experiments using both KCl and NH_4Cl, it is also possible that differences between the NTS electrophysiology and the behavior exist because the gustatory system was altered over the course of behavioral testing. Perhaps prolonged experience with NH_4Cl amplified signals from afferents that synapsed with cells containing ammonium-sensitive receptors. Such a mechanism would not be observed in electrophysiological tests in which the rat's first experience with NH_4Cl was

on the test day. Furthermore, the subjects in these preparations are usually deeply anesthetized, allowing for little influence from higher brain regions on gustatory activity, unlike in an awake animal. Along these lines, stimulation of the central amygdala, thought to be important for attention and memory, has been shown to affect responses in the NTS and PBN (Li et al., 2002; Lundy & Norgren, 2001).

Another difference between the electrophysiological literature and the majority of the behavioral literature involves the depletion state at the time of testing. In order to motivate animals to perform behavioral tasks, researchers must often restrict food or water access, or deplete subjects of some other commodity like sodium. These methods are not necessary for electrophysiological recordings. The few electrophysiological experiments that have been reported using depleted animals have suggested that such a state can affect neural responses in some areas of the gustatory system. For example, sodium depletion, or hormones associated with depletion, affect both NTS and CT responses to NaCl (Contreras & Frank, 1979; Herness, 1992; Lundy, 1998; McCaughey & Scott, 2000; Tamura & Norgren, 1997). Likewise, vasopressin, a hormone important for regulating fluid homeostasis, can also significantly increase inward Na^+ and proton currents in hamster TRCs (see Gilbertson et al., 1993).

A paper by Lundy & Contreras (1999) is one of the few studies to claim that KCl and NH_4Cl could potentially produce discriminable perceptions. The central thesis of this paper is that NaCl, KCl and NH_4Cl could perhaps be discriminated by the rat due to across fiber patterns shaped predominantly by the activity of 2 classes of neurons in the geniculate ganglion. Sodium chloride (NaCl)-specialists appear to synapse with taste cells containing amiloride-sensitive, apically-located ENaCs that are highly selective for

Na^+, but also permit passage of small amounts of K^+ and to a lesser extent NH_4^+. Hydrochloric acid (HCl)-generalists, on the other hand, respond well to all 3 salts, but show a more robust response to NH_4Cl than to the others. The remaining types of ganglion cells respond equally well to all 3 salts. This hypothesis is plausible, but given that the difference in NaCl-specialist activation with KCl and NH_4Cl stimulation is very slight, does not sufficiently address how high concentrations of KCl would be distinguished either from low concentrations of NH_4Cl or from other nonsodium chloride salts and acids which produce moderate activation of HCl-generalist cells. Additionally, the finding that NH_4Cl and KCl are discriminable even in the presence of amiloride (Geran et al., 2002), when responses from NaCl-specialists should be suppressed, suggests that we must look elsewhere for the underlying cause of this discrimination.

As for the discrepancy among electrophysiological tests as to whether amiloride impairs nonsodium salt responsiveness of the CT nerve, this could be due to important differences in the methods employed. For instance, most of the experiments reporting inhibition with amiloride deposited a salt solution on the tongue immediately followed by either a mixture of salt and amiloride (Lundy & Contreras, 1997; Lundy et al., 1997), or amiloride alone (Minear et al., 1996). Thus, the nerve was adapted to the salt prior to amiloride treatment. When the reverse was attempted, Minear and colleagues (1996) noted that adapting the tongue to amiloride prior to salt treatment had no effect on KCl responding. This has also been shown to hold true for NH_4Cl responsiveness (Formaker & Hill, 1988; Hill & Bour, 1985; Hill et al., 1982). Also without effect were experiments in which the tongue was adapted to amiloride prior to the presentation of salt dissolved in amiloride (Brand et al., 1985). This method more closely resembles that of the current

detection threshold procedure. Rats ingest amiloride when they are reinforced for pressing the correct lever. This serves to adapt the tongue to amiloride prior to a stimulus trial consisting of salt dissolved in amiloride or amiloride alone. Differences among electrophysiological procedures also exist for duration of stimulus delivery, stimulus concentration, time constant, and whether the integrated response or the number of action potentials is used as the dependent variable, making comparisons difficult.

Implications for Chloride Salt Detectability

When the parameters from salt detectability functions measured to date were compared, we found that KCl was more similar to NaCl dissolved in amiloride than to NaCl dissolved in distilled water both in slope and threshold (Figure 5-1). Although, these functions are based on data from separate groups of subjects, this finding is interesting because NaCl + amiloride and KCl also share a similar taste quality (Hill et al., 1990; Kopka et al., 2000; Spector et al., 1996) and route of transduction at higher concentrations (Ye et al., 1994). Taken together, these results suggest that NaCl + amiloride and KCl are indiscriminable to the rat at a variety of concentrations due to activation of a common pathway. One caveat to this interpretation is that although the slope and threshold for KCl and NaCl + amiloride are similar, the asymptotic performance is significantly different. This is most likely due to the fact that rats in the amiloride experiment were trained on NaCl dissolved in distilled water, and therefore might have performed less well at the higher concentrations due to a change in perceived intensity or quality with amiloride at suprathreshold concentrations. Rats in the KCl experiment, however, were trained on KCl and therefore, presumably did not experience any changes in intensity or quality with testing. It would be interesting to test this

hypothesis by measuring NaCl + amiloride detectability in animals trained on a KCl vs. water task.

Given the results of the NaCl + amiloride experiment, it was hypothesized that the detectability function for NH_4Cl might also fit this pattern and support data from electrophysiological and taste quality experiments. Ammonium chloride shares a similar taste quality (Erickson, 1963; Hill et al., 1990; Morrison, 1967) and perhaps at least one transduction mechanism with KCl and NaCl + amiloride (DeSimone et al., 2001), but produces a detectability function that is different from that of KCl and instead very similar to that of NaCl (Figure 5-2), a stimulus that is easily discriminated from nonsodium salts at higher concentrations (Geran et al., 2002; Hill et al., 1990; Spector & Grill, 1992). In addition to evidence that NH_4Cl and KCl activate the same transduction pathway, or pathways (DeSimone et al., 2001), there is also evidence that NH_4Cl might utilize a separate AS pathway at lower concentrations (Kloub et al., 1997). If NH_4Cl transduction does depend on an AS route, this pathway cannot be blocked sufficiently by micromolar doses of amiloride to impair detectability. Larger doses of amiloride have not been tested as they might lead to nonspecific effects (see DeSimone & Ferrell, 1985).

Sodium chloride detectability appears to depend heavily on CT input. Precisely how heavily depends on the procedure, with values ranging from 1-2 $\log_{10}$ units for the shift following transection (Kopka & Spector, 2001; Slotnick et al., 1991; Spector, Schwartz & Grill, 1990). Kopka & Spector (2001) reported that the shift in threshold with CTX was not further impaired with amiloride treatment, suggesting that AS taste receptor cells innervated by the GSP are not important for normal NaCl detectability in the rat. Performance to concentrations greater than the mean postsurgical threshold of 0.1 M

NaCl are presumably due to combined activation of AI cells in the GL and GSP (Kopka & Spector, 2001). Potassium chloride detectability also appears to be largely dependent upon input from the CT. Chorda tympani transection increases KCl threshold by about 0.6 $\log_{10}$ units (Geran et al., 1999) to approximately 0.1 M KCl. This shift is similar to that observed for NH_4Cl, but much less than that reported for NaCl after CTX (Kopka & Spector, 2001; Slotnick et al., 1991; Spector, Schwartz & Grill, 1990). The KCl threshold for intact animals, however, is markedly (~ 0.5 to 0.7 $\log_{10}$ unit) higher than that of either NaCl or NH_4Cl. Thus, KCl and NaCl result in approximately the same threshold following CTX while the threshold for NH_4Cl is slightly lower (Table 5-1). This suggests that the remaining taste receptor cells innervated by the GL and/or GSP are more sensitive to NH_4Cl than to KCl or NaCl. This finding is supported by the electrophysiology literature in that each of the 3 main gustatory nerves responds more robustly to mid-range and high concentrations of NH_4Cl than to similar concentrations of KCl or NaCl (see Frank et al., 1983; Kitada et al., 1998; Sollars & Hill, 1998). It is not apparent, however, if this comparatively large response to ammonium is also observed at lower, near-threshold concentrations.

It is not known what effect, if any, the anion might have on salt detectability. To date, detectability has been measured for only 1 nonchloride salt (NaG) in the rat and it was found to be very similar to NaCl (Geran & Spector, 2000b). It is unclear whether other nonchloride salts like potassium gluconate or ammonium hippurate would also produce detectability functions that mimic those of their halogenated counterparts. Likewise, the detection thresholds of divalent salts like calcium and magnesium chloride have not been tested. In the future, it might be worthwhile to expand the number of stimuli tested and

nerve transections performed, including GSP and GL neurotomies, to better assess processes necessary for normal salt detectability in the rat.

Support for the Hypothesis That The Seventh Cranial Nerve Is More Important For Taste Recognition and Discrimination Than The Glossopharyngeal Nerve

A variety of nerve transection experiments have suggested that the gustatory branches of the 7th cranial nerve appear to be involved in taste recognition and discrimination, while the 9th cranial nerve, or GL, is more important for the expression of oromotor reflexes to aversive stimuli (see St. John & Spector, 1998). The discrimination experiments have involved tastants from each of the 4 main taste categories. These discriminations have included sucrose vs. maltose, citric acid vs. quinine, KCl vs. quinine, NaCl vs. KCl (St. John, Markison, Guagliardo et al., 1997; St. John & Spector, 1997; St. John & Spector, 1998; Spector & Grill, 1992; Spector et al., 1997) and now KCl vs. NH_4Cl and NaCl vs. NH_4Cl. Furthermore, although the GL is the only gustatory nerve that contains fibers highly responsive to quinine (Frank, 1991) and innervates approximately 60% of the taste buds in the oral cavity (Miller, 1995), GL transection has little to no effect on quinine performance in a variety of tasks. These include concentration-dependent avoidance in a 2-bottle preference test and detection threshold (Akaike et al., 1965; St. John & Spector, 1996). Additionally, in their seminal paper, St. John & Spector (1998) reported that GL transection likewise had no effect on KCl vs. quinine discrimination, while combined transection of the CT and GSP nerves did. This was surprising given that the GL contains fibers that are differentially responsive to these 2 stimuli while neither the CT nor the GSP are thought to contain a substantial number of quinine-sensitive units (Frank et al., 1983; Sollars & Hill, 1998), although a single fiber analysis of the GSP has not yet been performed. These results were interpreted as

evidence that input from the CT could potentially be important for the perception of a wide variety of taste qualities, not just the salt and acid stimuli to which it is most responsive.

This apparent dichotomy between the functional roles of the facial and GL nerves is more pronounced in the catfish (Finger & Morita, 1987). For these animals, the facial nerve is necessary for locating food while the GL is necessary for initiation of the appropriate oromotor response once a food stimulus has been located (see Caprio et al., 1993). In rodents, the role of the GL is not quite so obvious. Deficits in the sensory/discriminative and hedonic domains of taste function (see Spector, 2000), although minimal, can be produced with GL transection in addition to changes in oromotor reflexes. For instance, GL transection has produced a modest impairment in the quinine avoidance of naïve rats (Markison et al., 1999). Combined transection of the CT and GL increases the detection threshold for quinine and compromises pre-trained quinine avoidance in a brief-access test, although GL or CT transection alone is without effect on these tasks (St. John et al., 1994; St. John & Spector, 1996). This suggests that perhaps the GL and CT carry information that is redundant for the maintenance of these tasks in pre-trained rats. Deficits in aversive oromotor reflexes, on the other hand, are quite pronounced with GL transection (Travers et al., 1987), suggesting that the GL is more important for unconditioned reflexes to aversive stimuli (see Eylam et al, 2000), than for taste perception or hedonic responses.

While it is easy to appreciate the utility of fibers narrowly tuned for “bitter” compounds or salts and acids in performing such a function, it is less obvious why this nerve would contain fibers that are narrowly-tuned for sugars (Frank, 1991). It has been

hypothesized (St. John & Spector, 1998) that although the GL is not sufficient to maintain a KCl vs. quinine discrimination for rats trained while intact, this input may be sufficient for rats to learn this or other discriminations over time based on cues mediated by the GL. A second hypothesis concerning the role of the GL, is that this nerve could provide information important for determining suprathreshold stimulus intensity. Humans report an increase in perceived intensity of quinine following bilateral anesthesia of the CT nerve that appears to be due to a release of inhibition (see Catalanotto et al., 1993). It is possible that such a mechanism also exists in the rat, but has not been apparent due to obstacles inherent in measuring suprathreshold intensity in animal subjects. Clearly more research is necessary to determine the role of the GL in the taste-guided behavior of rodents.

Conclusions

Overall, this series of experiments supports the possibility of a labeled line for sodium taste quality in the rat, as the AS transduction pathway appears to be both necessary and sufficient for the recognition and detection of sodium salts (see Bernstein & Hennessy, 1987; Geran & Spector, 2000b). The fact that amiloride does not seem to affect the taste quality or intensity of KCl or NH_4Cl lends credence to the hypothesis that this AS pathway is appreciably activated only by sodium and lithium ions. In contrast, the perceived intensities of all three salts tested to date (i. e., NaCl, KCl and NH_4Cl) have been compromised by CT transection, suggesting that this nerve might be highly sensitive to low concentrations of both sodium and nonsodium chloride salts and/or provide input to regions of the gustatory CNS important for salt detection. It would be interesting to test this hypothesis using a wider array of salt stimuli and nerve transections.

Also of interest is the finding that KCl and NH_4Cl, stimuli that produce similar patterns of activity in the NST (Erickson, 1963), are discriminated by the rat. This result raises questions about the effect of training on the tuning characteristics of the NST, as well as the possibility that across-fiber pattern coding, as currently applied, might not always be the best method for taste quality classification. Furthermore, this finding underscores the importance of using several different behavioral tasks to describe the perception associated with a particular taste stimulus and suggests that perhaps small differences at the neural level might produce significant differences at the behavioral level. Perhaps focusing on neural and behavioral differences within a prototypical taste quality, such as "saltiness," might enable researchers to make interesting predictions about the coding of a particular class of taste stimuli or even taste coding in general.

Table 5-1. Mean Detection Thresholds for Intact and Chorda Tympani-Transected Rats

Stimulus	**Mean Threshold for Intact Rats**	**Shift in Threshold with CTX**	**Mean Threshold for CTX Rats**
NaCl [a]	~ 0.006 M	1.0 -1.2 $\log_{10}$ units	~ 0.1 M
NaCl + 100 μM amiloride [a]	~ 0.04 M	0.4 $\log_{10}$ units	~ 0.1 M
KCl [b]	~ 0.03 M	0.6 $\log_{10}$ units	~ 0.1 M
NH_4Cl	~ 0.009 – 0.01 M	0.5 $\log_{10}$ units	~ 0.04 M

[a] Values from Kopka & Spector, 2001.
[b] Values from Geran et al., 1999.

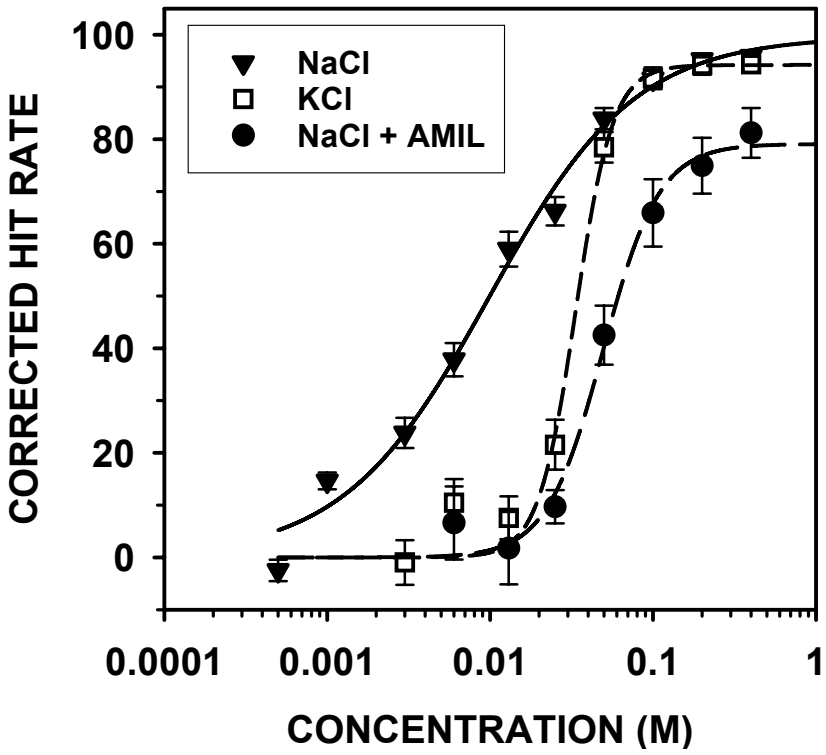

Figure 5-1. Comparison of NaCl and KCl Detectability Functions. Note that the slopes and inflection points (thresholds) are very similar for KCl (open squares) and NaCl + amiloride (closed circles), but not for NaCl (closed triangles). Both of the NaCl functions are from Geran & Spector, 2000a while the KCl function is from a separate group of rats (Geran et al., 1999).

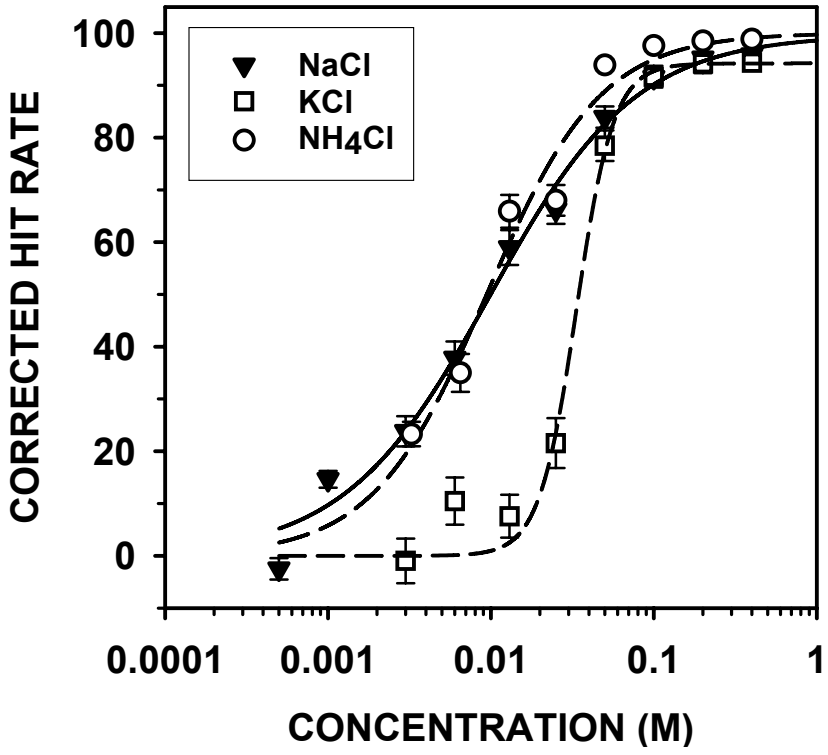

Figure 5-2. Comparison of Chloride Salt Detectability Functions. Note that the slopes and inflection points (thresholds) are very similar for NaCl (closed triangles) and NH_4Cl (open circles), but different for KCl (open squares). The NaCl and KCl functions are from Geran & Spector, 2000a & Geran et al., 1999.

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BIOGRAPHICAL SKETCH

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