Gustatory neural processing in the brainstem of the rat
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CHAPTER 1

General Introduction
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to a study on gustatory neural processing in the brainstem of the rat

1. Preface

The work presented in this thesis is the result of neuroanatomical and physiological investigations on the gustatory system of the rat. It focuses on the identification of gustatory neurons, their projection patterns and their possible influence on autonomic output. This first chapter summarizes briefly the relevant knowledge about gustation, the characteristics of gustation, the physiology and neuroanatomical organization of the gustatory system, both peripherally and centrally, and finally the physiological and neuroanatomical background of viscerogustatory interactions in response to ingestion. The final paragraph formulates the aim and outline of the thesis, followed by a summary of results.

1.1 Gustation; A Special Visceral Afferent System

The chemical sense of taste is (together with olfaction) the most primitive of specialized sensory systems, with an evolutionary history of some 500 million years. Accordingly, it deals with one of the most fundamental requirements of life necessary to sustain the individual: feeding.

The sense of taste evaluates the acceptability of a chemical in the mouth and guides the decision to ingest or reject. A decision to swallow is final, so the organism must select wisely from its chemical environment. While the organism must not accept toxins, it cannot afford to bypass nutrients. Diverse nutrients are called for, and the optimal mix may change with age, disease and nutritional or reproductive status. Rats and other animals do select a proper diet over time. Therefore taste responses are influenced not only by chemicals that are taken into the mouth, but also by past experiences and the momentary physiological status of the organism. So, the sense of taste manages dietary selection not only by its analysis of the quality and intensity of potential food substances, but also through communication with the visceral senses.

Gustation should be seen as intermediary between the physical sensory systems and visceral sensation. It possesses certain features of nonchemical senses, such as identification of stimulus quality and intensity through spatio-temporal codes. Yet, taste may be classified as a visceral afferent system according to its embryological, anatomical and functional characteristics shared
with the other afferent neurons of the cranial parasympathetic nerves that innervate the body cavity. The location of gustatory receptors at the transition between the external and internal environment also supports the classification of taste as a special visceral afferent system.

1.2 Characteristics of the Sense of Taste

1.2.1 Primary Taste Qualities

Perception of food is a complex experience based on multiple senses: taste per se, olfaction, somesthesis, thermoreception and nociception. Taste qualities are commonly divided into four categories or primary taste qualities: sweet (represented by sucrose), sour (represented by HCl), salty (represented by NaCl) and bitter (represented by quinine-HCl). However, the concept of four basic qualities is questionable, since psychophysical data have shown that various sapid substances did not fall within the range spanned by the four qualities. Among these is umami (Japanese for the specific taste sensation elicited by monosodium glutamate), which is qualitatively different from any of the four basic tastes. Furthermore, data from conditioned taste aversion paradigms clearly show inter- and intra-species differences concerning the perception of taste quality. As early as 1916, Henning proposed the terms "taste quality continuum" or "taste space", because four tastes obviously do not account for the whole range of taste sensitivities.

1.2.2 Taste Dimensions

For physical sensory systems like vision or audition, one quality dimension is determined by stimulus wavelength. The presence of a rational stimulus dimension urges the experimental approach to deciphering the particular sensory system. On the contrary, taste dimensions are very hard to define. In spite of the absence of obvious stimulus dimensions, particular taste qualities are known to be determined by a number of stimulus characteristics. These include pH, hydrophobicity, molecular weight and configuration. However, they fail to relate to the entire range of taste sensation. Moreover, molecules that elicit similar verbal reports of taste from humans or neural activity in gustatory afferent nerves often bear little chemical relation to one another. For example, many mono- and disaccharides taste sweet to humans. Not all sugars however, taste particularly sweet, but many other unrelated molecules such as glycine and alanine, do so. Thus, the choice of stimulating chemicals and their concentrations remain a theoretical
issue, rather than a simple starting point for analysis. For sensory data, various multivariate statistical analyzes have been used to describe stimulus relationships. Among these are, hierarchical cluster analysis (which may show the presence of different clusters of stimuli and attributes each stimulus to a cluster) and multidimensional scaling (which may show interrelationships between different stimuli) are often used to map the distinguishability of data.

1.2.3 Gustatory Neural Coding

The neural coding of taste qualities in vertebrates is addressed by the labeled line and the across-fiber pattern theories. The basic differences between these theories are as follows. The labeled line (LL) model proposes that each taste quality is transmitted via a separate 'neural line' through the medulla, thalamus and cortex. This model assumes that individual gustatory fibers are more or less narrowly tuned to one of the primary taste qualities and that the function of any one neuron would be to signal its particular encoded taste quality. The across-fiber pattern (AFP) model proposes that individual afferent fibers lack absolute specificity. The afferent neuronal message for quality is expressed in terms of the relative amount of neuronal activity across neurons or across classes of neurons. These two theories reflect two completely different ways of looking at the central nervous system. The LL model emphasizes a single neuron's activity, as is common in many attempts to elucidate CNS functioning by the single-unit recording technique. The AFP model, on the other hand, emphasizes the importance of patterns of neuronal activity, occurring and changing continuously in the CNS. Gustatory neural coding may utilize both types of mechanisms.

1.3 Anatomy and Physiology of the Peripheral Gustatory System

1.3.1 Taste Buds and Taste Receptor Cells

In vertebrates, taste is perceived through specialized epithelial cells, taste receptor cells, which are organized within discrete ovoid clusters: taste buds. Taste buds are found within the oral cavity, embedded in the epithelium of the tongue. Focal collections of taste buds are termed taste papillae. The fungiform, circumvallate and foliate papillae are found on the anterior two-thirds of the tongue, the posterior tongue and the lateral edge of the tongue, respectively. Mammals also have scattered non-papillar taste buds that populate the soft palate, larynx, pharynx, epiglottis and the upper part of the esophagus. A typical vertebrate taste bud contains 50 - 100 elongated receptor cells with microvillar
processes extending into an apical taste pore. Mature taste receptor cells have a life span of 10 - 14 days and are continually replenished from precursor cells, found at the base of the taste bud.

Taste stimuli are dissolved during mastication and reach the apical microvilli of taste cells by mixing and diffusion through saliva. Solutes in the oral cavity make contact with the apical membranes of the taste receptor cells via the taste pore, but are shielded from direct contact with the lateral and basal aspects of the taste cells, which are connected via tight junctions. Saliva is an aqueous mixture of electrolytes, proteins (enzymes), etc., whose composition is variable and largely a function of parasympathetic stimulation. From the view of taste transduction, saliva plays a role in depolarization of taste receptor cells in response to taste stimulation.

There is a significant amount of lateral connectivity between taste receptor cells within a bud; both electrical and chemical. It appears that the taste bud functions as a signal processor to sum and shape taste responses from the interconnected taste receptor cells.

1.3.2 Taste Transduction
Taste receptor cells are secondary receptor cells, i.e. electrically excitable cells not capable of generating action potentials. Direct interaction of taste stimuli with apically located ion channels mediates the transduction of monovalent salts, sour and some bitter components. Voltage-dependent channels for Na+, K+, and Ca2+ have been shown to be present in taste receptor cells. Specific membrane receptors appear to be required for the transduction of amino acids, sweet stimuli and some bitter-tasting compounds. There are at least two ligand-gated cation channels for the amino acids arginine and alanine and a glutamate gated channel which responds to monosodium glutamate in taste receptor cells. Sweet and bitter taste are transduced by second messenger mediated pathways. Both sweet and bitter compounds are thought to bind to specific G-protein coupled receptors, and activate the second messengers cAMP and IP3, respectively. Recently McLaughlin and Margolskee cloned a G-protein, gustducin, that is specifically expressed in gustatory tissue and may play a role in sweet and bitter taste transduction.

1.3.3 Cranial Nerves
The sensory fibers that innervate the taste receptors travel in cranial nerves VII, IX and X to form synapses in the nucleus of the solitary tract (NTS) in the medulla.
The greater superficial petrosal nerve and the chorda tympani nerve (CT) carry fibers from the facial nerve (VII) to innervate taste buds on the palate and the anterior portion of the tongue (fungiform), respectively. Buds within the circumvallate papillae and the posterior portion of the foliate papillae are innervated by the lingual branch of the glossopharyngeal nerve (IX) and the taste buds of the larynx, pharynx, epiglottis and esophagus are innervated by the superior laryngeal branch of the vagal nerve (X). Several papillae are innervated by branches from the same fiber and one papilla is innervated by branches originating from several fibers.\(^{110}\)

Apart from conveying gustatory information, taste fibers are known to interact among one another. In gerbil, lateral inhibition between chorda tympani nerves was observed, which may function to reduce spurious sensory signals in taste axons.\(^{146}\) Furthermore, chorda tympani nerve fibers are known to modulate ion transport and thereby possibly taste transduction, across lingual epithelium in dog.\(^{171}\)

In rodents, the cranial nerves that carry gustatory axons from the oropharyngeal region penetrate the lateral medulla in fascicles, traverse the spinal trigeminal tract and nucleus, collect in the solitary tract and terminate in an overlapping rostrocaudal order within the NTS.\(^{5,86,191}\)

### 1.3.4 Peripheral Chemosensitivity

Sensitivity to the four classical taste qualities is distributed variably among receptor cells. A number of researchers have recorded intracellular responses of taste receptor cells to chemical stimulation.\(^{131,157}\) They all showed that single cells responded to more than one taste quality. In the rat only 10 to 17% of the taste receptor cells respond exclusively to one of the four taste compounds used. The remaining cells respond to two, three or all four chemicals.\(^{131}\)

The same lack of specificity is found among taste buds and papillae, although taste buds in different oral regions display different best responses to the four taste qualities.\(^{47,48,116}\)

As early as 1941, Pfaffmann showed that one gustatory fiber could be activated by several of the four stimuli and that the qualitative sensitivity determined for each fiber depends on the set of stimuli applied.\(^{133}\) However, the relatively effective chemical stimuli differentiate among the gustatory nerves and nerve branches. For example, in the rat CT there is one type of fiber that responds well to both acid and sodium.\(^{22,47}\) The response to sugars and to quinine is more species specific.
1.4 The Primary Gustatory Nucleus

The *nucleus of the solitary tract* (NTS), as found in mammals, birds, reptiles, amphibians and fish, comprises the primary sensory nucleus for both the gustatory and general visceral modalities. The NTS is a heterogeneous nuclear complex in the form of a long column, extending rostro-caudally from the pontomedullary junction to the caudal medulla.

1.4.1 Topographic and Chemotopic Organization

The NTS has three major subdivisions \(^97\), the rostral gustatory (rNTS), intermediate (iNTS) and caudal autonomic part (cNTS), which, in turn, are composed of several subnuclei. The iNTS, located at the level of the fourth ventricle and area postrema, separates the gustatory and visceral regions. The cNTS receives afferent innervation from all major organs in the body and, as such, is the major visceral sensory relay group in the brain. The cardiovascular, pulmonary, respiratory tract and gastrointestinal receptor afferents all project to specific areas in the caudal NTS \(^97\) and innervate the medial, interstitial, and the parvocellular subnuclei, respectively \(^7,42,71,97\). The gustatory NTS receives, from rostral to caudal, the central axonal processes of primary afferents from taste receptors located in the anterior two-thirds and posterior one-third of the tongue, the larynx, pharynx, epiglottis and esophagus \(^122\). Some somatosensory afferents from the oral cavity, head and face also terminate in the gustatory part of the NTS \(^67,96,102\) and the rNTS also receives intranuclear input from caudal NTS subnuclei \(^10\). In addition, the NTS receives considerable centrifugal input from forebrain structures (see 5.1, reciprocal connections).

Within the gustatory NTS a rough chemotopy of taste afferent terminations is present; this topographic organization is related to the chemosensitivity and peripheral spatial distribution of the gustatory afferents \(^68\). For example, although neurons responsive to anterior and posterior tongue stimulation are widespread within the gustatory part of the NTS \(^183\), anterior tongue responding neurons are generally located rostrally to posterior tongue responding neurons \(^203,204\). Despite their differential distribution, sucrose-activated neurons are distributed evenly along the medio-lateral axis of the NTS, while quinine-activated neurons are concentrated medially \(^68\).

1.4.2 Chemosensitivity

Units recorded in the NTS are not easily identified; they may be presynaptic
afferents, postsynaptic afferents or even interneurons involved in intranuclear data processing. Most units recorded are subject to this ambiguity. In two recent studies Nakamura and Norgren extracellularly recorded single-unit responses of NTS neurons in awake rats, after gustatory stimulation with the standard four taste stimuli and a number of additional stimuli that were chemically or behaviorally related to the standard stimuli. Taste neurons in the NTS most often responded best to sucrose (41%), less frequently to citric acid (30%) and NaCl (25%) and infrequently to quinine (4%). At the concentration used, 44.6% of the taste cells responded significantly to only one of the gustatory stimuli. Among these specific neurons, 51.1% was citric acid specific, 40% was sucrose specific, 6.7% was NaCl specific and 2.2% was quinine specific. By use of comparable data from unanesthetized preparations, they stated that the breadth of responsiveness (i.e., the frequency of occurrence of specific taste cells) remained virtually unchanged between the CT and the NTS neurons. Norgren and Nakamura also showed that taste neurons in behaving animals responded differently from those in anesthetized preparations. In awake animals NTS neurons responded more selectively to the four standard taste qualities, they displayed a higher mean spontaneous firing rate and the percentage of neurons that responded to only one stimulus, the so-called specific neurons, was much higher (34 vs. 5.5 %). Earlier experiments in anesthetized animals showed that second-order NTS cells are more broadly responsive compared to chorda tympani fibers as far as their sensitivity to the four standard stimuli is concerned. These contradictory results are probably due to anesthesia. Two other important factors that may account for differences that are found concerning the chemosensitivities of NTS gustatory neurons in different experiments, were discussed by MA and Erickson. First, the differences in application of gustatory stimuli (whole mouth stimulation, vs. stimulation of the anterior tip of the tongue by a tongue chamber) causes stimulation of different sets of taste buds. Second, they showed that the best stimulus labels of responding NTS neurons, which are usually determined by response magnitude, are considerably different for different analysis intervals. For instance, in their study, 23 of the 45 responding NTS neurons were labeled as bitter-best and 7 as acid-best in the first second, whereas in the 2-5 second interval one bitter-best and 18 acid-best labels occurred. These differences are caused by quality-specific differences in temporal response patterns.

Monroe and DiLorenzo showed that the chemosensitivity of NTS neurons may not only depend on their input, but may also be related to their projection target. Using gustatory stimulation of the tongue and extracellular single-unit
recording of NTS neurons in anesthetized rats, they found that gustatory NTS neurons that project to the pontine parabrachial nucleus (PBN) are most frequently acid-best, while neurons that do not project to the PBN are most frequently NaCl-best.

1.4.3 Sensory Processing

The fact that first-order gustatory fibers and second-order gustatory neurons closely resemble each other in their chemosensitivity, suggests that there is little sensory processing of responses past the first synapses in the gustatory pathway. However, there are various indications for the occurrence of neural processing of sensory information within the NTS. When compared to primary taste afferents, rNTS neurons show a higher spontaneous activity and, after gustatory stimulation of the tongue, display a different pattern of neuronal discharge and a higher spike frequency. Furthermore, there is extensive convergence of first-order taste fibers onto second-order neurons. For example, nerves innervating the anterior tongue and the palate converge on rNTS neurons. The same holds true for fibers innervating the anterior tongue and the nasoincisor ducts and for the quinine-evoked input of the glossopharyngeal and chorda tympani nerve. Activity of individual taste fibers might sum up, inhibit each other's actions or work synergistically, leading to neural processing in rNTS neurons. Finally, the different morphological, biophysical and pharmacological properties of gustatory NTS neurons also indicate evidence for considerable processing by the rNTS. rNTS neurons are not a homogenous set of cells. They can be separated into morphological groups based on visual inspection (multipolar or stellate, elongate or fusiform and ovoid cells) and on quantitative differences (number, length and the extent of branching of their dendrites). Neurons have also been separated into groups based on their intrinsic membrane properties, such as repetitive discharge patterns and ionic conductances. In addition, rNTS neurons receive multiple inputs and respond to both excitatory (glutamate, substance P) and inhibitory (GABA) neurotransmitters.

In sum, these results indicate that the rNTS is capable of considerable synaptic processing of afferent sensory information.

1.5 Central Gustatory Pathways

A common pattern of connectivity for the primary gustatory nucleus exists across all vertebrates. This involves an ascending (lemniscal and limbic) system, a local
reflex system and a descending visceral system that influences autonomic output. It is hypothesized, that only about 20% of NTS gustatory neurons contribute to ascending projections \(^{128}\) and that the remainder form medullary connections or contribute to autonomic output \(^{121}\).

1.5.1 Ascending Gustatory Pathways

Gustatory neurons from the NTS project largely ipsilaterally to the pontine parabrachial nuclei (PBN). Parabrachial gustatory cells send axons both to the ventral forebrain and to the thalamic taste relay. Thalamic taste neurons ascend and synapse in the insular gustatory cortex. Some PBN axons project directly to the insular cortex \(^{94}\); whether this monosynaptic projection is specifically gustatory remains unproven. The PBN taste neurons projecting into the ventral forebrain reach many more and larger neural structures, but the function of these nuclei is apparently not gustatory or even purely sensory. These parabrachial ventral forebrain projections include the hypothalamus, amygdala, preoptic area and the bed nucleus of the stria terminalis. These gustatory limbic connections are thought to be responsible for the affective and hedonic aspects of gustation, which guide and regulate feeding behavior \(^{135,136}\). The thalamocortical pathway is responsible for the associative aspects of gustation and the discriminative capacity of the taste system \(^{135,136}\). Details on the relevant structures are given in the following paragraphs.

\textit{PBN.} The ascending gustatory projections originate from the rostral, central division of the rNTS \(^{65,195}\) and project largely to the PBN. The PBN is known as the pontine taste area \(^{125}\), although this nucleus is involved in processing visceral sensory information as well. The functional-topographic specificity seen in the NTS is maintained in the PBN \(^{64,71}\), the lateral PBN subnuclei being the visceral-sensory parts and the medial subnuclei the gustatory parts. Projections from the gustatory NTS are found in the caudomedial parts of the PBN complex \(^{10,145,188,193}\), in the "waist area" and in the medial, external medial, ventral lateral and centrolateral PBN subnuclei \(^{71}\). In rat, the most intense taste responses were recorded rostrally in the medial PBN, just ventral to the brachium conjunctivum. Weaker responses were recorded within the "waist area" and within the ventral lateral and centrolateral PBN \(^{125}\). These responses resulted from sapid stimuli applied to the anterior tongue alone. The terminations within the external medial PBN have not been associated with taste function yet \(^{71}\), however, they project to and terminate in close vicinity of the thalamic taste relay \(^{201}\).
The parabrachial gustatory neurons display the same degree of chemo-specificity as second-order NTS neurons and first-order gustatory fibers. PBN neurons can be grouped based on a higher response to a preferential stimulus among four, but breadth of responsiveness does not change compared to NTS neurons or primary taste fibers.

**Thalamus.** Gustatory PBN projections to the thalamus are bilateral and terminate densely in the parvocellular part of the ventral posteromedial nucleus (VPMpc) and to a lesser extent in the parafascicular, central medial and other midline nuclei. This area was defined as a gustatory thalamic relay, although it represents a relay for all lingual sensory modalities, rather than just taste.

The response characteristics of thalamic taste neurons have been shown to differ in a number of ways from primary and secondary gustatory neurons. Chemical stimulation of the tongue may inhibit thalamic spontaneous activity, all four standard taste stimuli evoked responses of similar magnitude and only 20% of the thalamic gustatory neurons responded with increasing neuronal activity to increasing concentration of stimuli. These more complex response properties may assist in fine discriminations between related stimuli, but they might also participate in relating gustatory neuronal activity to other sensory events.

**Gustatory Neocortex.** The primary gustatory neocortex is situated immediately ventral to the somatosensory cortex and receives gustatory input from the PBN and VPMpc. So, the anatomical relationship between taste and somesthesis is fulfilled through the cortical level. After gustatory stimulation of the oral cavity, gustatory neurons were observed in the granular and dysgranular cortex; most of these cortical neurons responded to mechanical stimulation as well.

**Limbic projections.** The limbic gustatory pathway terminates mainly in the central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST). Weaker projections reach the lateral hypothalamus (LHA). These ventral forebrain projections originate predominantly from the PBN, but there are also some direct projections from the rNTS to the LHA and the paraventricular hypothalamic nucleus. Gustatory responses have been recorded from neurons in the LHA and CeA; however, these responses are
nor pure, nor plentiful. For instance, gustatory neurons in the CeA are responsive to tactile, thermal and chemical stimuli.

Reciprocal Connections. Both thalamocortical and ventral forebrain gustatory systems form reciprocal connections with the PBN and the NTS. In addition, the gustatory cortex projects heavily back to the thalamic taste relay.

The majority of these data are strictly anatomical and only a few studies document that the descending forebrain projections actually engage gustatory neurons. It seems nevertheless reasonable to assume that by reciprocal connections, a network is established by which factors such as motivation or conditioning can influence gustatorily mediated acceptance-rejection reflexes.

1.5.2 Local Medullary Gustatory Pathways

Neuroanatomical and neurophysiological studies have demonstrated that local medullary pathways link oral afferent nerves to oral motor nuclei, and that gustatory information may influence oral motor behavior. Electromyographic analyzes have shown that taste nerve sectioning can disrupt the patterns of motor activity associated with licking, swallowing and gaping. Furthermore, electrophysiological experiments showed that the medullary reticular formation, which receives rNTS input, contains neurons that are active during licking and swallowing. The hypoglossal motor neurons, innervating the tongue musculature, receive direct rNTS input. The facial and trigeminal motor nuclei which are responsible for muscle control of face, lips and jaw receive indirect gustatory input through the parvocellular and medullary reticular formation. Anterograde tracing studies have shown that neurons in the rostral central subdivision of the rNTS primarily contribute to ascending projections, but also project to the ventral part of the rNTS. Neurons in the ventral part of the rNTS give rise to intramedullary projections and represent the gustatory premotor outflow. So, a multi synaptic pathway links the gustatory afferent rostral central subdivision with the premotor cells in the ventral subdivision of the rNTS.

1.5.3 Descending Gustatory Pathways

The NTS subserves a major efferent function via regulation of autonomic mechanisms, including vagal parasympathetic and orthosympathetic control. The rostral parvocellular reticular formation receives direct input from the rNTS and projects to the preganglionic parasympathetic neurons of the superior salivatory
nucleus. Furthermore, the medullary reticular formation that receives rNTS input, directly projects to the preganglionic ortho- and parasympathetic neurons of the dorsal motor nucleus of the vagus (DMnX), the nucleus ambiguus (Amb) and the intermediolateral cell column. Whether these descending autonomic pathways are of gustatory origin remains to be established.

1.6 Viscerogustatory Interactions in Response to Ingestion

Ingestion may have physiological consequences (adverse or beneficiary) that become associated with the prior taste experience. In turn, this association may influence later ingestive behavior. This association implies the occurrence of gustatory and visceral interactions. Gustation is closely and reciprocally related to metabolic factors associated with ingestion. Neuroanatomically seen, the NTS is well situated to integrate gustatory and visceral related consequences. The rNTS receives afferent gustatory information, while the majority of the caudal half receives input from the gastrointestinal tract. In addition, hepatic vagal afferents terminate in the medial subdivision of the left NTS. Although direct connections between gustatory and viscerosensory regions of the NTS have not been established yet, taste cell dendrites extend into the viscerosensory NTS and a viscerogustatory link through the medullary reticular formation has been demonstrated.

There are at least two possible ways in which gustatory information may interact with visceral afferent and efferent responses. First, general visceral inputs may alter gustatory responses and feeding behavior. Physiological experiences caused by ingestion, (such as illness), continuously accommodate the gustatory code. The physiological condition of the animal induces short-term fluctuations in gustatory sensitivity, which promote or inhibit feeding and encourage consumption of a nutritionally replete diet. Second, gustatory information may change neuronal responses to general visceral receptor input or induce changes in autonomic output. This would mainly be established by the occurrence of cephalic reflexes that anticipate the digestion and often even the ingestion of food.

1.6.1 Visceral Influence on Gustatory Responses

*Experiences.* The gustatory experiences of suckling rats establish preferences that persist into adulthood. Preferences also develop through association of taste with positive reinforcement, particularly with a visceral reinforcement such as occurs with the administration of a nutrient of which the animal has been
deprived. Gustatory preferences may even be established in humans and other animals by mere familiarity or through constant exposure. However, the most potent effect of experience on subsequent preferences occurs at the development of a conditioned taste aversion (CTA). This is a form of associative learning, in which animals or humans react aversively to the taste of a food that has previously been paired with illness. Robust aversions can be acquired after a single pairing of a taste (conditioned stimulus; CS) with a drug-induced (often LiCl) illness (unconditioned stimulus). Chang and Scott have recorded modified neural responses to gustatory stimulation with the CS in the NTS of the rat after establishment of a CTA. Other studies showed changes of NTS neuronal activity by changes in c-fos expression in the NTS to a CS before and after conditioning.

Several investigators have demonstrated that both permanent and temporary lesions of the gustatory PBN severely impair, if not abolish, the rat's ability to acquire a CTA. However, it remains to be established whether the proposed deficit in taste-visceral integration is the result of damage to the PBN neurons themselves or to their projections to the forebrain. Several experiments have demonstrated that lesions in certain forebrain structures cause deficits in the acquisition of a CTA. For example, lesions of the gustatory neocortex, central amygdala, lateral hypothalamus or the ventroposterior medial (gustatory) thalamus impair the ability of rats to acquire a postoperative CTA. Recent studies have shown that in rats with a lesioned NTS acquisition and retention of a CTA was still possible. These results demonstrate that the rNTS is not essential for the chemical identification necessary for CTA learning.

Physiological Condition. The physiological condition of an animal is closely related to its choice of foods. Compensatory feeding behavior that may occur as a response to deprivation of specific nutrients results from a taste-directed change in food selection. It is presumed that the recovery from nutrient deficiencies is paired with the taste that preceded this recovery. In this way a conditioned taste preference is established, by which the hedonic value of the taste is enhanced. This implies that the hedonic value of a taste experience must be subject to changes.

Specific food preferences appear to result from deficiencies, such as cravings for thiamine, threonine or histidine. In many species, depleting sodium stores of the body triggers an innate appetite for sodium. During such an episode concentrations of sodium salts that are normally rejected are avidly ingested. This compensatory response to the physiological need for salt results
from a change in the hedonic value of tasted sodium \(^{34,40}\). Jacobs \textit{et al.} \(^{78}\) recorded gustatory NTS neurons in sodium-deprived and normal rats and demonstrated a profound depression in responsiveness of salt-oriented neurons and a sharp increase in activity in sweet-oriented cells. They suggested that the perceived quality of sodium in sodium-deficient rats was shifted from salty to sweet. If rats restore depleted sodium levels following deprivation, the responsiveness of gustatory NTS neurons returns to a predeprived state \(^{107}\).

The expression of sodium appetite following acute NaCl depletion is eliminated by PBN lesioning \(^{46}\). Supracollicular decerebration experiments (rostral to the PBN) have demonstrated to impair concentration-dependent NaCl intake, in contrast to sucrose intake \(^{45}\). This implies that the brainstem mechanisms are not adequate for the control of sodium intake. This is not due to an inability to taste the stimulus. Rather, decerebration appears to disrupt the stimulatory effects of postabsorptive feedback, provided by the NaCl solution related to hydrational needs \(^{45}\). The neuronal mechanisms controlling hydrational balance have been localized to the forebrain \(^{21,74,173}\). Hydrational factors and not taste factors, seem to stimulate the ascending portion of sodium intake functioning \(^{45}\).

Satiety, mimicked by gastric distension was demonstrated to selectively depress gustatorily induced taste responses in the NTS of the rat \(^{57}\). The greatest effect on activity was evoked by sucrose, followed by NaCl and HCl; the responses to quinine-HCl were unmodified. Relief from distension reversed this effect within a 45 min period.

Signals from gastric mechanoreceptors are carried by the vagus nerve and might act on rNTS neurons through the PCrt \(^{50,72,219}\). The involvement of both the NTS and DMnX in gastric distension has recently been demonstrated by c-fos expression \(^{213}\). However, other results suggest that gastric distension affects gustatory activity through the release of factors that inhibit gastric emptying. This phenomenon is not dependent on gastric vagal afferents \(^{53}\).

While changes in amino acid or sodium levels are appreciated over several days, the availability of certain macronutrients, specifically sugars, is of more immediate concern. Giza and Scott and Giza \textit{et al.} \(^{54,56}\) demonstrated that exogenous administration of glucose, insulin or glucagon suppress multi-unit activity evoked from gustatorily responsive NTS neurons to lingual application of glucose, NaCl and HCl in the rat. They hypothesized that chemicals increasing glucose availability may promote satiety for palatable taste substances by reducing afferent activity in gustatory neurons. Psychophysical studies \(^{28,29}\) and common experience showed that a decrease in gustatory responsiveness has a
perceptual counterpart. Perceived glucose intensity declines and the decrease of hedonic appeal promotes meal termination.

There are several possible mechanisms through which circulating glucose, insulin or glucagon might exert their actions on gustatory responsiveness.

Firstly, this may be brought about by stimulation of gastrointestinal and hepatic chemoreceptors and subsequent activation of the vagus nerve. Visceral information is projected to the NTS through vagal activation. It has not been demonstrated yet, that first-order gustatory and visceral vagal afferents converge upon NTS neurons. Neuroanatomical and electrophysiological experiments have demonstrated overlapping projections from hepatic (vagal) and gustatory regions of the NTS within the immediate subjacent parvocellular reticular formation (PCRt) as well as in the PBN. Herewith, the PBN and PCRt emerge as the first brainstem nuclei in which significant integrative processing of gustatory and visceral afferent information occurs.

Secondly, gustatory neurons in the NTS might be influenced by feedback from forebrain structures associated with feeding. The hypothalamus and central amygdala are both associated with hedonic mechanisms accompanying ingestion. Aside from possible centrifugal contributions to changes in taste activity, several of these forebrain structures have been shown to possess glucose, insulin and glucagon sensitive sites, through which they might exert their actions on gustatory NTS neurons. However, several lines of evidence suggest that the brainstem independently mediates ingestive responses to glucoregulatory challenges, thereby excluding the involvement of forebrain structures.

The third mechanism that may alter gustatory responsiveness is a direct action on NTS neurons through glucoreceptors and insulin receptors sensitive to endogenous glucose and insulin levels, respectively. Caudal NTS activity has been demonstrated to be modifiable by iontophoretic application of glucose and insulin. These activity changes might directly affect the rNTS or reach the rNTS through the PCRt. Insulin and glucose may also act on the area postrema, which passes on the signal to the gustatory NTS.

Finally, gustatory responsiveness might be altered through the release of catecholamines, somatostatin or growth hormone. Up to now these effects have not been evaluated.

1.6.2 Gustatory Influence on Visceral Responses

Cephalic Phase Responses. Cephalic phase responses (CPRs) are autonomic and endocrine reflexes that are triggered by sensory contact with food, rather than
by postigestional consequences of food \cite{26,105,138}. The digestive effects that result from cephalic reflexes are initiated most reliably by the taste and smell of food. The sight of food and other circumstances associated with eating may also act as stimuli, although these seem to be less potent. The cephalically stimulated responses are rapid (generally occurring within minutes after sensory stimulation), small (relative to the magnitude achieved when food is actually being metabolized) and transient (returning to near-baseline levels within minutes) \cite{105}. Cephalic reflexes include the secretion of saliva \cite{132}, release of gastric juices \cite{81}, pancreatic enzymes and insulin \cite{26}. Other, less studied CPRs are changes in gastric motility, hepatic control of blood glucose and bile flow \cite{138}. CPRs have been examined in man, monkey, dog, cat, sheep, rabbit and rat \cite{1,138}. In functional terms, the CPRs make the gastrointestinal tract ready to move, digest and absorb food. They prepare the viscera to metabolize and store nutrients. In sum, CPRs act as feedforward mechanisms to inform the organism about the ultimate postigestional consequences of food. CPRs can be evoked by normally ineffective stimuli, once they have been paired several times with an inherently effective stimulus, as in a classical conditioning paradigm \cite{14,215}. The gustatory afferent contribution to CPRs, therefore, cannot always be asserted.

The most frequently studied CPR is the cephalic phase insulin release (cPIR) or preabsorptive insulin release, by the endocrine pancreas \cite{16,139,177,181}. After oral intake of glucose, insulin is released by the endocrine pancreas within one minute, before the blood glucose level starts to rise \cite{33,61,177}. The cPIR is vagally mediated through efferent parasympathetic fibers that innervate the pancreatic β-cell \cite{119}, since it can be blocked by atropine \cite{137} and vagotomy \cite{18,26,141}. Much research has been done on the localization of the vagal preganglionic and the identification of the vagal branches that mediate the cPIR. Powley and Berthoud \cite{17,18,140,141} have shown that the perikarya of the vagal preganglionic controlling the response are located in the medial columns of the DMnX. These neurons send their axons through the two hepatic and gastric branches of the abdominal vagus. Each of these branches can independently mediate a cPIR.

Although lesions of the ventromedial hypothalamus (VMH) \cite{15,49,178}, CeA \cite{151} and the lateral hypothalamus (LH) \cite{60,178} alter the cPIR, the local circuitry of the brainstem seems to be sufficient to elicit the cPIR, since cephalic insulin release persists in decerebrated animals \cite{43}. The CNS integrating pathways of the cPIR are not fully defined. Higher diencephalic brain centers (including the hypothalamus) seem to exert a modulatory role on the main brainstem relay systems \cite{12,142}, rather than being the exclusive site for the integration of gustatory afferent information.
and efferent control over pancreatic β-cells. Indeed, Hayama et al.\textsuperscript{69} showed that the neural structures above pre- or midcollicular levels have tonic inhibitory or facilitatory influences on response properties of extracellularly recorded NTS taste units after oral stimulation with the four classical taste stimuli. This modulatory role may determine several aspects of the cPIR. Glucose elicits a potent cPIR, but it does no longer do after it has been paired with LiCl injection in intact rats\textsuperscript{14}. CTA does not seem to be a capacity of the chronic decerebrate rat\textsuperscript{62}, so the modification of the cPIR in CTA may also require the forebrain.

2 Aim and Outline of the Study and Summary of Results

The aim of the present thesis is to determine the neuroanatomy and physiology of gustatory information processing in the brainstem of the rat. It focuses on the neuroanatomical background of viscerogustatory interactions in response to ingestion, with special attention to the cPIR. In the experiments we describe the main focus is on the brainstem. CTA and sodium appetite following NaCl deprivation are examples of viscerogustatory interactions which require information processing with forebrain structures associated with feeding. However, the brainstem is capable to influence ingestive behavior on the basis of taste information, independently from forebrain nuclei. For example, the neural circuitry of the brainstem mediates ingestive responses to glucoregulatory challenges and is sufficient to induce the cPIR, thereby excluding the involvement of forebrain structures.

Gustatory input activates parasympathetic reflexes (such as the cPIR) to anticipate and to support the digestive process. The pathway that conveys gustatory information to the endocrine pancreas should involve the rostral NTS, where primary, gustatory afferents terminate. Since these gustation-induced reflexes are vagally mediated, the DMnX should also be part of this descending parasympathetic pathway (Fig.1). The aim of the present thesis is to demonstrate which brainstem connections are essential to elicit the cPIR and how specific their involvement is in this response. Accordingly, the study combines neurobiology with metabolic physiology.

In chapter 2 experiments are described in which a retrograde transneuronal viral tracer (pseudorabies virus ,PrV) was injected into the endocrine pancreas, to demonstrate descending projections conveying gustatory information. The major advantage of viral tracing is the visualization of functional chains of neurons. The results of this study did not substantiate a direct connection between the rNTS and
the DMnX. Therefore, we suggested the involvement of one or more intermediate

stations. Based on the viral tracing results, we proposed the intermediate or caudal NTS, the medullary reticular formation and the PBN as candidates to complete this pathway (Fig. 1).

For the studies described in chapter 3 we used c-fos immunocytochemistry to detect sweet taste-activated neurons in the brainstem. Several studies have demonstrated a tight correlation between neuronal activity and the expression of c-fos\textsuperscript{154,169}. In this experiment rats drank a sucrose solution which acted as a stimulus to induce c-fos expression and the subsequent production of Fos protein in transsynaptically activated neurons\textsuperscript{154}. Sucrose taste-activated neurons involved in the processing of gustatory information were detected in the

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**Figure 1.** Schematic drawing summarizing possible pathways in the brainstem to connect oral/pharyngeal/esophageal taste receptors to DMnX areas innervating the pancreas.
intermediate NTS, caudal DMnX and in the medial and lateral PBN, which are known to process information related to ingestive behavior.

In chapter 4 we again applied the c-fos technique in rat brainstem, after intra-oral infusion of sucrose and saccharin and intra-gastric infusion of sucrose. During the infusion, we took several blood samples to analyze plasma glucose and insulin levels. This experimental design enabled us to discriminate between Fos synthesis caused by sweet taste and by the postingestive effects following ingestion of a sweet tasting solution. The results demonstrated that the NTS is also activated by visceral afferents conveying information about glycemia and through direct activation of NTS glucoreceptors by circulating glucose. Sweet taste information inducing a cephalic phase response is probably responsible for the remaining c-fos expression in the NTS. Neuronal activation by sweet taste information inducing the cPIR is reflected in the vagal preganglionic neurons of the medial columns in the DMnX of saccharin-infused animals.

Chapter 5 describes the intramedullary projections of the rNTS, which were anterogradely traced with Phaseolus vulgaris Leucoagglutinin (Pha-L). rNTS projections on hypoglossal motoneurons and ‘premotor’ cells in the medullary reticular formation provide neuroanatomical evidence for taste influence on oral motor behavior. Gustatory influence on parasympathetic reflexes, such as the cPIR, was found to reach the DMnX through the medullary reticular formation or through intra-NTS projections to the intermediate NTS.

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