Published in final edited form as:

J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 2008 February ; 194(2): 135–143. doi:10.1007/s00359-007-0276-0.

Sorting Food from Stones: The Vagal Taste System in Goldfish, *Carassius auratus*

Thomas E. Finger

Dept. Cell & Developmental Biology, Univ. Colorado Medical School, Aurora, CO 80045

Abstract

The sense of taste, although a relatively undistinguished sensory modality in most mammals, is a highly developed sense in many fishes, e.g. catfish, gadids, and carps including goldfish. In these species, the amount of neural tissue devoted to this modality may approach 20% of the entire brain mass, reflecting an enormous number of taste buds scattered across the external surface of the animal as well as within the oral cavity. The primary sensory nuclei for taste form a longitudinal column of nuclei along the dorsomedial surface of the medulla. Within this column of gustatory nuclei, the sensory system is represented as a fine-grain somatotopic map, with external body parts being represented rostrally within the column, and oropharyngeal surfaces being represented caudally. Goldfish have a specialization of the oral cavity, the palatal organ, which enables them to sort food particles from particulate substrate material such as gravel. The palatal organ taste information reaches the large, vagal lobe with a complex laminar and columnar organization. This lobe also supports a radially-organized reflex system which activates the musculature of the palatal organ to effect the sorting operation. The stereotyped, laminated structure of this system in goldfish has facilitated studies of the circuitry and neurotransmitter systems underlying the goldfish's ability to sort food from stones.

Keywords

palatal organ; vagal lobe; nuc. solitary tract; nucleus ambiguus; ingestion

Introduction

The tradition of comparing the overall body plan of various organisms is ancient, dating back to the early scientists of the ancient civilizations. A resurgence occurred during the 17th Century when naturalists in Britain and elsewhere began compiling drawings of diverse life forms (e.g. Samuel Collins; see Kruger 2004). The more formal field of comparative neurobiology had its roots in the end of the 19th Century with the early neuroanatomists including Cajal and Ariens Kappers. The field flourished in the early 1900's in the hands of the Herricks, J. B. Johnston and their students. One of the key concepts pervading comparative neurobiology is that by letting nature perform the experiments of species and niche specializations, scientists can learn which features of a system are fundamental features common to many forms, and which traits are derived specializations or elaborations on the common plan. This paper describes one of the specializations on the bauplan of the gustatory system.

The sense of taste evolved in the earliest vertebrates. Taste buds, although absent in hagfish, can be recognized in lampreys, fishes including chondrichthyes, amphibians and amniotes. A key feature of the taste system is that taste buds are always innervated by one of the cranial nerves that contain ganglion cells derived from epibranchial placodes (CN VII: facial; CN IX glossopharyngeal; CN X: Vagus) (Barlow and Northcutt 1995) although the taste bud cells themselves arise from the local epithelium (Barlow and Northcutt 1995; Stone et al. 1995).

In all vertebrates, the taste nerves enter the medulla to terminate within the visceral sensory column as described by Herrick (Herrick 1922) and others. These nerves also carry general visceral information, e.g. from heart or gut, but the general visceral inputs terminate in separate, more caudal reaches of the visceral sensory column. This general pattern of organization occurs in all vertebrates and thus must be regarded as part of the overall “bauplan” of the brain.

In amniote vertebrates, the gustatory system is relatively staid and varies little across species. In contrast, the relative size and complexity of the gustatory system varies greatly between teleosts. At least 5 groups of fishes appear to have elaborated different types of complexities in this system all the while maintaining the basic bauplan.

This paper will focus on the highly elaborate vagal gustatory system in the common goldfish, *Carassius auratus*. In goldfish and closely related carps, the brainstem nuclei serving vagal-mediated taste functions can occupy upwards of 20% of the total mass of the brain (Kotrschal and Palzenberger 1992). This is clearly an important system in these species.

Casual observation of goldfish, as many of us do when we are children, admits of no obvious complex behaviors that may be served by such an elaborate neuronal machine. Indeed, my own initial observations of goldfish led me to the conclusion that they barely have any behavior whatsoever. If one sprinkles food along the top of the water, the fish pick off individual food particles, apparently using vision in a fashion similar to almost all other diurnal animals. But if one waits for the food to sink to the bottom, a more interesting and subtle behavior can be observed. The food tidbits mix in with the substrate – gravel, sand or mud. The fish orients head downward and vacuums up the mixture. The fish then manipulates the mix in the mouth, finally spitting out what was just taken in. Such is the appearance. But more careful observation shows that what the fish spits out is only the substrate; all the food has been retained. This task is tantamount to our placing a small candy into a handful of fine gravel and popping the whole handful into one's mouth. The task then is to sort out the candy from the stones. We can do this, albeit slowly, and by using a serial consideration of each potential object in the mouth. Goldfish use a different strategy. In order to understand this behavior, it is necessary to understand the structure of the oral cavity of a goldfish. This description below is based on the elegant work of F. Sibbing (Sibbing et al. 1986; Sibbing and Uribe 1985) who used SEM and X-ray cinematography to study the oral cavity and food handling of a carp which is similar in structure and function to goldfish. A more recent work by Callan and Sanderson confirms and extends these findings (Callan and Sanderson 2003).

The Mouth of a Goldfish

The oral cavity of a goldfish is divisible into anterior and posterior regions (Fig. 1b). The anterior section, the orobuccal cavity, is relatively simple with a hard palate forming the roof and an uncomplicated floor. Along with the lips, the anterior oral cavity functions something like a straw or simple tube to bring the substrate and food into the posterior oral cavity. The posterior oral cavity (also called the anterior pharynx) of goldfish and carps is specialized compared to other fishes and contains the apparatus necessary for the delicate food-sorting behavior.

The posterior part of the mouth of a goldfish is characterized by the muscular palatal organ which is something like a tongue attached to the roof of the mouth. Although the palatal organ cannot be protruded grossly like a human tongue, the organ is nonetheless highly muscular. Based on studies on closely related carps (Sibbing and Uribe 1985; Callan and Sanderson 2003), the function of the palatal organ appears to be to locally protrude downward to trap food particles against the rigid gill arches and gill rakers that form the floor of the oral cavity (See Fig. 2b). The degree of motor control of the organ is remarkably fine since goldfish are capable of sorting out finely-ground food particles from a bad-tasting gelatin matrix (Lamb and Finger 1995). The exact biomechanics of this organ are not well understood, but local activation of the muscles results in discrete protrusions outward from the face of the organ.

The essential feeding behavior of goldfish and related carps is to suck up substrate, food, etc. into the oral cavity, array the mouthful in the space between the palatal organ and the gill arches, and then to trap food particles between the palatal organ and branchial apparatus that forms the floor of the oral cavity. By means of backwashing and rinsing, the fish can then retain the food particles while ejecting the inorganic substrate material (Callan and Sanderson 2003; Sibbing et al. 1986).

Carps (and by extension, goldfish) use several different strategies to select food from substrate depending on the density and sizes of the different objects (Callan and Sanderson 2003). With slurries and fine substrate material, the fish can use the gill rakers and branchial apparatus as a crossflow filter, i.e. the heavier particles fall out of the main flow passing between the gill arches, while the lighter material (i.e. food slurry) remains in the main flow to move rearward in the oral cavity and pharynx (Callan and Sanderson 2003; Sanderson et al. 2001)(see Fig. 2a).

When the fish must separate larger objects, e.g. food pellets from gravel, an entirely different strategy is employed. A casual observer can watch a goldfish suck up a mouthful of gravel from the bottom of a tank, apparently chew on it (while manipulating the food and substrate particles into position in the oral cavity) and then spit out the gravel that the fish just picked up. What can't be observed easily is that at the same time the fish is rejecting the inert substrate material, the goldfish has retained the majority of the organic material. This behavior can, however, be seen using intraoral endoscopy (Callan and Sanderson 2003). The fish then moves the presumptive food materials posteriorly for re-sorting or ultimately chewing in the pharyngeal grinding organ. All of these feeding-related behaviors appear to have a gustatory component. Both the palatal organ and pharynx are replete with taste buds, and taste cues are the primary determining factor as to whether a fish will reject or swallow a potential morsel (Lamb and Finger 1995; Sibbing and Uribe 1985).

Neural Substrates of Food-Sorting

Both the sensory and motor innervation of the palatal organ involve the vagus nerve. The palatal organ is richly innervated by an anterior component of the vagus nerve which is independent of the general visceral branch of the nerve that innervates the viscera including esophagus, heart, and digestive tract. A separate, large anterior vagus ganglion lies between the brain and the underlying palatal organ. Major nerve trunks extend from this ganglion into both the palatal organ and the more laterally-situated gill arches. Thus this ganglion contains all the cell bodies of ganglion cells that provide innervation to the food sorting organs: palatal organ and branchial apparatus. Motor roots providing innervation to the palatal musculature course through and beside the ganglion before entering the medulla. The sensory and motor roots that provide innervation of the palatal organ and gill arches form a distinct large cranial nerve root that enters the ventrolateral aspect of the medulla. At this level lies an unusual structure, even by

fish standards. The medulla of a goldfish is dominated by the vagal lobe, which is a large, layered structure amounting to nearly 20% of the volume of the entire brain (Kotrschal and Palzenberger 1992). Grossly, it is the same size as the optic tectum in this species (see Fig. 3a).

Vagal Lobe

The vagal lobe is a complex, derived structure which contains both a primary sensory nucleus and motoneurons. The primary sensory nucleus is homologous to a part of the nucleus of the solitary tract; the motoneurons are derived from a part of the nucleus ambiguus of other vertebrates. The nucleus of the solitary tract receives primary taste inputs from the facial, glossopharyngeal and vagus nerves. The vagal lobe corresponds only to the vagal gustatory portion of this complex. The nuc. ambiguus contains alpha motoneurons that innervate branchial arch musculature of which the palatal musculature is the main component.

Laminar Organization

The vagal lobe is separated into 3 major layers, from outside in: the sensory layer, a central fiber (white matter) layer and a deep motor layer. The primary sensory fibers enter the lobe through the central fiber layer and turn outward to end in the sensory layer. Likewise, the motor root takes its origin from motoneurons which lie within the motor layer and send their axons ventrally to exit as the most medially-situated fibers of the nerve root (see Fig. 4).

The sensory layer of the vagal lobe is itself divisible into sublaminae alternating between layers of cells and layers of neuropil (Fig. 3c; Morita and Finger 1985; Morita et al. 1980). Selective filling of the vagal nerve roots shows that the nerve branches that innervate the palatal organ end in different sensory sublaminae than do the nerves innervating the gill arches. Palatal sensory nerves end uniquely in layer VI, while branchial nerve fibers end in layer IV. The two systems converge onto layer IX (Fig. 3C). In addition, a separate superficial root contains sensory fibers – perhaps non-gustatory sensory fibers – that end in the outmost layers of the lobe. Thus the different laminae of the lobe contain the sensory representation of the two opposing oral surfaces: the roof of the mouth in layer VI and the floor of the mouth in layer IV.

Orotopy

Neural tracer injections into the feeding-related organs of the oral cavity reveal a further level of organization to the system. Small injections into the palatal organ result in labeling of only a small region of neuropil within the sensory layers of the vagal lobe. Lateral injections label dorsal areas of the lobe; midline injections label ventral patches of neuropil (Fig. 3B). Similarly, anterior injections in the palatal organ label the anterior part of the lobe while injections in the posterior palatal organ label areas of posterior vagal lobe. What's more, the representation of the gill arches appears in a corresponding position within the lobe, i.e. posterior gill arches are represented posteriorly, while anterior arches are represented anteriorly. The vagal lobe then maintains an orotopic map – i.e. the neural representation of the oral cavity within the lobe is mapped according to spatial location in the oral cavity. A similar orotopic map occurs in the much smaller vagal lobe in catfish (Kanwal and Caprio 1988).

The orotopy of the goldfish vagal lobe is not limited to the sensory layers. Injections of tracer into the palatal organ retrogradely label motoneurons in the vagal lobe motor layer as well as areas of neuropil in the sensory layers. A limited injection into the palatal organ labels only a few motoneurons and these always lie directly radially inward from the area of labeled sensory neuropil. So the vagal lobe contains a sensory map and a motor map each in register with the other.

A Gustatory-mediated Reflex

Can this in-register mapped anatomical organization underlie the intraoral food-sorting behavior of these animals? The basic behavior of the fish is to retain the larger morsels that activate the taste system in an appetitive fashion. To effect this behavior, the palatal organ needs to activate the local palatal muscles to force a downward protrusion of the palatal organ to trap the food particles between the roof and floor of the mouth (Callan and Sanderson 2003).

A simple circuit that could mediate this behavior would be a local reflex loop from each point in the sensory layers to the corresponding pool of motoneurons that innervate the palatal muscles inserting at that point in the organ. One such simple circuit is shown in Fig. 4b. In this model, the incoming sensory activity: 1) terminates in a restricted region of the sensory layers of the lobe, 2) activates second-order neurons that extend axons radially inward to terminate in the motor layer, where they 3) activate local motoneurons which effect the local protrusion of the palatal organ.

Our experiments show that the essential elements of this simple circuit do exist within the vagal lobe. Small injections of tracer into the sensory layers reveal a limited, local projection from the sensory to the motor layers (Fig. 5). Conversely, small injections of tracer into the motor layers retrogradely label a column of bipolar neurons in the sensory layer situated radially outward from the injection site (Goehler and Finger 1992). The radial orientation of the dendrites of the reflex interneuron emphasizes the predominant radial organization of the vagal lobe sensory layers (see also Morita et al. 1980).

The neuroanatomy of the system then is consistent with the simple hypothesized reflex circuitry. Primary afferent fibers terminate on second-order neurons of the sensory layers which in turn synapse onto neurons of the underlying motor layer. The motor layer, however, contains GABAergic interneurons as well as primary motoneurons. Does activation of the neurons of the sensory layer in fact lead to excitation of underlying motoneurons? Our studies in *in vitro* slice preparations indicate that the answer is yes. Electrical stimulation of local areas of the sensory layers (as would happen when a small piece of food comes into contact with the palatal organ), leads to activations of only those motoneurons lying immediately inward from the area of activation (Ikenaga et al. 2007).

Neurotransmitters in the Vagal Lobe

Our *in vitro* studies of this system demonstrate that glutamate is the key neurotransmitter both for the primary gustatory afferent fibers and for the reflex interneurons of the lobe. In both situations, the glutamate acts on postsynaptic ionotropic glutamate receptors (both AMPA/kainate and NMDA) to activate the post-synaptic cells. The situation in relationship to the primary afferent fibers is the best studied and perhaps the most interesting from the neuroethological perspective.

Primary Gustatory Afferents

Electrical stimulation of the primary afferent fibers evokes a multiphasic response in the sensory layers as revealed by extracellular field potentials (Finger and Dunwiddie 1992; Smeraski et al. 1999). Responses to a single shock stimulation of the nerve are blocked nearly completely by DNQX or other inhibitors of AMPA/kainate receptors. The response to a stimulus train is, however, more complex. The response to each stimulus following the first gets progressively larger over a period of 50-100 ms following the first stimulus. The enhanced response is mediated by NMDA receptors which initially are blocked by Mg^{++} . Once the

Mg⁺⁺ block is removed by the initial depolarization, the NMDA component becomes apparent. Similarly, in Mg⁺⁺-free conditions, the underlying NMDA component is readily apparent even in the presence of DNQX (Smeraski et al. 1999). So the response to primary afferent stimulation is initially mediated by AMPA/kainate receptors, but upon repetitive stimulation involves a larger NMDA component.

The NMDA component may serve an important function in terms of integration of the afferent input. The NMDA-mediated response could serve either as a temporal or spatial filter. As a temporal filter, the enhanced NMDA response could serve to separate scattered single spikes from a burst of action potentials within the primary afferent network. That is, the large NMDA response to a spike train would be useful in distinguishing response bursts from single spikes in a relatively noisy primary afferent network. However, the few recordings made from individual gustatory afferent fibers in other species of fish reveal a fairly low rate of spontaneous firing with pronounced bursts upon stimulus presentation (Konishi and Zotterman 1961)

Another role of NMDA receptors on the second-order neurons could be for spatial integration of inputs onto the dendrites. Behaviorally, what is important to the fish is when a food particle is in contact with both the palatal and branchial surface of the oral cavity. In this situation, activation of the palatal musculature will sandwich the food particle between the two surfaces. If the food contacts only the palatal surface, then activation of the palatal muscles is unlikely to effect capture of the particle since it will not be resting on the opposite surface. Since the inputs from the branchial and palatal surfaces terminate in 2 different sublaminae of the sensory layer (respectively layers IV and VI), then only when these layers are activated simultaneously and in register, will the particle be positioned for effective capture. A neuron with NMDA receptors and a dendrite extending through both of these layers will be well-situated to give an enhanced response to co-activation of synapses within these two layers. This is exactly the situation of reflex interneurons in the vagal lobe. The reflex interneurons have both NMDA receptors and dendrites extending through the two primary afferent layers. Thus near-coincident activation (within 50-100 ms) of the palatal and branchial afferents arising from the same part of the oral cavity should maximally activate the post-synaptic reflex interneurons. (Fig. 6)

Reflex System

The in vitro slice preparation also permits study of the neurotransmitters utilized by the interneurons linking the sensory layers to the motor output of the vagal lobe. As described above, reflex arc within the vagal lobe is topographically ordered so that each segment of the motor layer receives input only from those interneurons situated immediately outward from those motoneurons. In other words, for a given area of the vagal lobe, the sensory input is linked to the motoneurons that activate the muscles controlling the part of the palatal organ that provides the sensory input to that particular region. This can be appreciated in preparations in which the motoneurons are retrogradely labeled by the calcium indicator, calcium green dextran, and where we can electrically stimulate different parts of the sensory layer. Motoneurons are activated maximally only from those parts of the sensory layer that are directly radially outward from the motor pool. These physiological findings mirror the anatomical findings of limited radial connectivity of the system. (Fig. 7)

Pharmacological manipulation of these slices shows that AMPA/kainate and NMDA receptors underlie the reflex activation of the motoneurons of the motor layer. The AMPA/kainate antagonist DNQX blocks the fast evoked response of the motoneurons, but a residual NMDA-mediated component can be seen in Mg⁺⁺-free Ringer's even in the presence of DNQX. Some complexity is, however, present in this system in that numerous small GABAergic (Glutamic

acid decarboxylase-positive) neurons also are situated in the motor layer and are likely to modulate the local activity of the motoneurons.

Complexities and Unknowns

While the model we present above has the advantage of being simple, unfortunately, it is also simplistic. The behavior is far more complex than the simple “where it tastes good, hold on” paradigm described above. First, the fish has far more behavioral sophistication than this model presupposes. The feeding strategy and therefore palatal movements are very different depending on the size and nature of the food (Callan and Sanderson 2003). Goldfish have complex oropharyngeal movements depending on the type of food being ingested (Callan and Sanderson 2003; Sibbing et al. 1986). Second, there is likely to be a strong tactile component to the sensory signal. The gustatory nerves give robust tactile as well as chemical responses (Kanwal and Caprio 1988; Konishi and Zotterman 1961). Yet a tactile signal alone is not sufficient to trigger the selective local activation of the palate as food particles do (Callan and Sanderson 2003). Finally, the gustatory system of goldfish signals not only appetitive cues, but also aversive ones. Food pellets doped with quinine, caffeine and other aversive compounds trigger rejection not ingestion (Lamb and Finger 1995). Thus at least the sign of a taste stimulus, if not the exact quality, must be encoded in the incoming taste signal. We have no information on the representation of quality in the vagal lobe. Possibilities include representation in different layers or sublaminae, representation in different functional columns within the sensory layers, or differential patterning of the incoming signal.

The relatively simple feeding behavior of goldfish has allowed us to begin a dissection of the underlying neural components. Many of the properties of this elaborate, derived system are identical to features of the gustatory system in mammals. For example in both rodents and goldfish, transmission from primary gustatory afferents relies on glutamate acting on ionotropic receptors on the second-order neurons. Once again, the study of a model system has allowed for a better understanding of general principles of brain organization as well as of the elegant, simple complexity of nature.

A Remembrance of Theodore Holmes Bullock

My first awareness of Ted Bullock came as a graduate student when I ran across two great compendia summarizing much of what was known about the organization of the nervous system in the middle of the 20th Century. The first of these, “*The Comparative Anatomy of the Nervous System of Vertebrates Including Man*” known by the names of its authors: Ariens Kappers, Huber and Crosby (1936), summarized the opening decades of the emergence of rigorous comparative studies of the brains of vertebrates. The second great compendium of the day was “*Structure and Function in the Nervous Systems of Invertebrates*” referred to as Bullock and Horridge (1965). I knew that the authors of the vertebrate book were either dead or long-since emeritus and so I envisioned the authors of the invertebrate tome to be similarly decrepit. Naturally, I was quite surprised not long after to find that Bullock was writing exciting papers on electric fish. “Maybe it’s his son” I thought. But no, that seemed wrong; how many Theodore Holmes Bullocks could there be? But he’s an invertebrate guy, I thought; what’s he doing working on fish? This was the first important lesson I learned from Ted (although he wasn’t there at the time): Just because you work on a particular system or with a particular technique should not limit either your curiosity or the direction of your research.

Not long after this, my mentor, Harvey Karten, announced that he had arranged a sabbatical in Ted Bullock’s lab, and wouldn’t I like to come along.? Of course I went. We worked on the neuroanatomy of electric fish; I on catfish and Len Maler on the weakly electric South American fish. We often lunched with Ted and his lab at the Scripps snackbar, and spoke of

electric fish, and anything else that came to mind. Ted Bullock proved to be accessible, congenial, gracious and vibrant – not at all the ancient, brittle figure I had envisioned back in the graduate school library.

Years later, I returned to Ted's lab for a mini-sabbatical of my own to study electrosensory processing in the diencephalon of catfish. I had identified anatomically an area I thought should receive lateral line or acoustic input and suggested to Ted that we could explore it using electrophysiology (for which I was ill-equipped in my own lab). Of course, he was enthusiastic about the plan and welcomed me to a space in his lab. We discovered an auditory and mechanosensory nucleus of the diencephalon and published a short article describing our findings (Finger & Bullock, 1982). I am grateful to Ted not only for his enthusiastic hospitality, but for another great lesson he taught me: The world is full of wonderful forms of life each has an interesting story to tell, if only you take the time to listen.

Acknowledgements

The author thanks the many collaborators and colleagues whose studies contributed to understanding the vagal lobe. Several of the summary diagrams in this work were adapted from work of Takanori Ikenaga. The experiments described herein comply with the "Principles of animal care", publication No. 86-23, revised 1985 of the National Institute of Health, and were approved by the Univ. Colorado Health Sciences Center Inst. Animal Care and Use Comm..

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Figure 1.

(a) A goldfish in a posture typical for benthic feeding. The fish sucks up material from the bottom and then sorts food from inorganic material by means of an elaboration of the palatal musculature and branchial apparatus. (b) SEM views of the roof and floor of the mouth of a carp (adapted from Sibbing and Uribe, 1985). Food particles are trapped between the palatal organ and the branchial apparatus while inorganic materials are flushed from the mouth.

Figure 2.

Schematic drawings of how the palatal organ and branchial apparatus separate food from substrate under different conditions. (a) When food particles are small enough to be suspended in the water, the gill arches serve as a crossflow filter (Callan and Sanderson 2003). As water passes across the gill arches, the heavier particles, e.g. sand, fall out while the lighter food (small red crosses) is carried rearward toward the posterior pharynx. (b) A sequence (1-3) showing how larger food particles and larger substrate particles (e.g. gravel), food are actively sorted by trapping the food particles between the palatal organ and the floor of the mouth. (1) The food particles activate taste buds on the palatal organ and gill arches (red lines). (2) This causes reflex protrusion of the palatal organ to press the food particles against the gill arches

and hold them in place. (3) Backwashing then results in expelling the inorganic matter out of the mouth or through the spaces between the arches.

Figure 3.

(a) The head of a goldfish opened to show the brain and innervation of the gill arches. The vagal lobe is a large protuberance from the medulla which is unusual even among fishes. The vagus nerve roots issue from the base of the vagal lobe to fan out into the gill arches. Deep vagal roots also penetrate the palatal organ. A general visceral branch of the vagus nerve, typical of all vertebrates, is barely visible as it courses caudally and deep to the branchial and palatal roots of the vagus nerve. The larger and more obvious posterior lateral line nerve (not labeled in this figure) runs caudally across the exiting roots of the vagus nerve. (b) A cross section through the head of a goldfish showing the relationship between the vagal lobe and the food-sorting apparatus of the oral cavity. Large vagal nerve trunks extend ventrally from the vagal

lobe to innervate the palatal organ. The orotopic organization of this system is indicated on this panel by the numerals showing corresponding points between the oral cavity and their representation in the vagal lobe. (c) Laminar organization of the sensory layers of the vagal lobe. This micrograph shows the alternating bands of termination of the vagus nerve sensory fibers. Inputs from the palate end in layers VI and IX; inputs from the gill arches terminate in layers IV and IX.

Figure 4.

(a) Micrograph of the vagal lobe showing the overall laminar organization. The sensory fibers terminate superficially in the sensory layer; the motor output to the palate originates from motoneurons of the motor layer. (b) Hypothesized reflex circuit utilized in food-sorting. Sensory information from a particular point in the oral cavity will terminate in a limited portion of the sensory layer (1) where it activates local interneurons (2). These interneurons have axons that traverse the fiber layer to terminate on the underlying motoneurons (3). The motoneurons that receive the reflex connection innervate muscles in the same local region of the palate from which the sensory input arises.

Fig. 5. Micrograph of the dorsal part of the vagal lobe showing the existence of the circuit shown diagrammatically in Fig. 4b. A small tracer injection was placed into the sensory layer and labels axons that run perpendicular to the layers of the lobe and end in and around the underlying motoneurons (Labeled Terminals). The bundle of labeled efferent fibers that comprise the ascending secondary gustatory tract also can be seen in the deep portion of the fiber layer (c.f. schematic diagram in Fig. 4b).

Fig. 6.

Hypothesized circuitry underlying activation of second-order neurons in the reflex pathway. The primary sensory fibers release glutamate which acts on ionotropic glutamate receptors – both AMPA/kainate and NMDA type -- of the interneurons. The NMDA receptors can act as spatial integrators and coincidence detectors for those neurons with dendrites passing through both layer IV and layer VI. These two layers receive differential input from the palate and gill arches. Because of the NMDA receptors, only when a particle activates both inputs within 50-100ms of each other would the interneuron be maximally stimulated. The traces shown at the right side of the figure compare the sizes of evoked field potential without activation of NMDA receptors (above) and with activation of NMDA receptors (below) by repetitive stimulation of primary gustatory afferents (From Smeraski et al. 1999). Near co-incident activation of layer IV and VI afferents would be expected to have a similar effect of magnifying the postsynaptic response.

Fig. 7. Schematic diagram of the reflex circuitry in the vagal lobe (modified from T. Ikenaga). The primary gustatory fibers (red) utilize glutamate as the neurotransmitter which acts on NMDA and non-NMDA ionotropic receptors on the post-synaptic cells of the sensory layer (orange and purple). Axons from these interneurons pass through the fiber layer to reach the motor layer where they terminate on both motoneurons (green and orange) and small GABAergic interneurons (blue) of this layer. These sensory layer interneurons utilize glutamate as a transmitter which acts on NMDA and non-NMDA receptors on the motoneurons which in turn activate the palatal musculature.