SHORT COMMUNICATION

Bimodal (Taste/Tactile) Fibers Innervate the Maxillary Barbel in the Channel Catfish

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Abstract

Analysis of single fibers isolated from a branch of the facial/trigeminal complex innervating the maxillary barbel of the channel catfish, *Ictalurus punctatus*, indicated the existence of bimodal (taste/tactile) fibers. Of the 60 single fibers recorded, 14 (23%) responded to both taste (amino acid) and tactile stimulation, 43 (72%) were responsive to only tactile stimulation and three (5%) responded only to taste stimulation. Quinine hydrochloride at a concentration of ≥1.0 mM suppressed the mechanosensory activity of the bimodal fibers, but had no effect on the tactile-only fibers. **Chem. Senses 22: 477–482, 1997.**

Introduction

Gustation and somatosensation are intricately involved in orosensation in vertebrates. The lingual branch of the trigeminal (V) nerve innervates oral regions and is implicated, along with the gustatory nerves, in ingestive behaviors (Zeigler *et al.*, 1984; Berridge and Fentress, 1985). Facial (VII), glossopharyngeal (IX) and vagal (X) cranial nerves innervate taste cells and process gustatory information from different regions of the oropharyngeal cavity, whereas the trigeminal (V) nerve processes general oral skin sense information. The close association between gustation and somatosensation in orosensation is clearly observed by the phenomenon of the 'taste localization illusion', whereby taste sensations are not localized to the specific locations of taste buds on the tongue, but to areas touched in the mouth (Todrank and Bartoshuk, 1991).

Although the gustatory cranial (VII, IX and X) nerves and the lingual branch of the trigeminal (V) are involved in the processing of chemical information (Silver and Finger, 1991), the trigeminal nerve is the major somatosensory input from oral regions to the central nervous system (CNS). The separate input of gustatory and trigeminal mechanosensory fibers to the primary gustatory nucleus of the medulla has been previously observed (Marui and Funakoshi, 1979). In accordance with this and with the anatomical studies showing in both fish (Kiyohara *et al.*, 1986) and rodents (Contreras *et al.*, 1982; Hamilton and Norgren, 1984) that gustatory and trigeminal (V) neurons converge onto medullary 'taste' nuclei, electrophysiological recordings in fishes (Marui and Caprio, 1982; Marui *et al.*, 1988; Smith and Marui, 1989; Lamb and Caprio, 1992, 1993) and amphibians (Smith and Marui, 1989) indicated that the majority of taste relay neurons recorded within the CNS were bimodal, responding to both taste and tactile stimulation. In addition, glossopharyngeal taste fibers in mud puppy (Samanen and Bernard, 1981), frog (Yamane, 1978) and sheep (Bradley *et al.*, 1983) were reported to be bimodal. Thus, the origin of the bimodality of CNS units may be the result of convergence of taste and tactile units and/or input by bimodal peripheral fibers. The present study in catfish addresses directly the question of the bimodality of 'taste' fibers, previously indicated to occur in the rat (Kasahara and Kawamura, 1975; Matsuo *et al.*, 1995).

Because of their highly acute sense of taste for amino acids, fish are excellent experimental models for the study of vertebrate gustation (Marui and Caprio, 1992). Multiunit recordings in fish indicate that facial, glossopharyngeal and vagal nerves respond vigorously to both taste (amino acid) and tactile stimulation (Davenport and Caprio, 1982; Kanwal and Caprio, 1983; Peters *et al.*, 1987; Marui and Caprio, 1992). Although taste stimuli alone can initiate food search in fish (Herrick, 1904; Bardach *et al.*, 1967), a concomitant tactile stimulus may enhance this action by both lowering the behavioral threshold (Hazlett, 1971; Thorington and Hessinger, 1988) and sharpening the animal's ability to localize the stimulus (Todrank and Bartoshuk, 1991). A quantitative analysis of single facial nerve fibers that innervate the anterior palate of the puffer fish, *Fugu pardalis*, indicated that chemosensory and somatosensory fibers were mutually exclusive (Kiyohara *et al.*, 1985). Of the 106 single palatine (facial) fibers of the puffer tested with five taste stimuli (5 mM HCl, 1 mM UMP, 10 mM L-proline, 0.5 M NaSCN and 0.5 M $CaCl_2$) and tactile stimulation, 34% were unimodally mechanosensitive and 66% were exclusively chemosensory. These results, however, are in direct contrast to indications from recordings from the recurrent facial nerve in the channel catfish that suggested the existence of bimodal (taste/tactile) fibers (Davenport and Caprio, 1982). Although bimodal taste/tactile fibers appear to be common for vertebrate glossopharyngeal (Yamane, 1978; Samanen and Bernard, 1981) and vagal (Bradley *et al.*, 1983) taste nerves, the relative percentage of bimodal facial taste fibers is unknown. Of 70 chorda tympani fibers recorded in the rat, 21 (30%) were mechanosensory and nine of these 21 (i.e. 13% of the total) were bimodal (Kasahara and Kawamura, 1975). A more recent study in the rat indicated that only four (6%) of the 65 single chorda tympani fibers were bimodal and 18 (28%) were mechanosensitive only (Matsuo *et al.*, 1995).

Twenty-five channel catfish, *Ictalurus punctatus*, 30–70 g, immobilized with an i.m. injection of Flaxedil (gallamine triethiodide; 0.05 mg/100 g body wt), were wrapped in wet tissue paper and positioned on a wax block in a Plexiglas container. Aerated artesian tap water containing the anesthetic, MS-222 (0.05% ethyl-*m*-aminobenzoate methane sulfonic acid), continually bathed the gills throughout the experiments. Supplemental Flaxedil and MS-222 were administrated as required. The electrophysiological preparation was similar to that described previously (Michel and Caprio, 1991); the exception was that in the present experiments, a section of polyethylene tubing only partially enclosed the barbel. Briefly, subsequent to deocculation, the peripheral cut end of a branch of the facial–trigeminal complex innervating the maxillary barbel was separated with fine forceps and glass pipettes into single and few fiber preparations in the orbit of the eye. The neural activity was recorded with a platinum–iridium hook electrode, a.c. amplified, monitored aurally, displayed on an oscilloscope and recorded on video tape. Data were analysed off-line with a computerized data acquisition and analysis system (Brain Wave Systems Discovery package; DataWave Technologies Corporation, Longmont, CO). The Brain Wave data files were exported as ASCII files for counting action potentials using BASIC-language programs developed by Dr Rainer Voigt (Boston University Marine Program, Woods Hole, MA).

Upon dissection of the maxillary barbel nerve into a single- or a few-fiber preparation, both chemical and tactile [both punctate and gliding (Biedenbach, 1971)] stimulation were tested. These tests occurred whether or not spontaneous activity was evident. Tactile stimulation was applied with a glass capillary tube (1.5 mm diameter) that

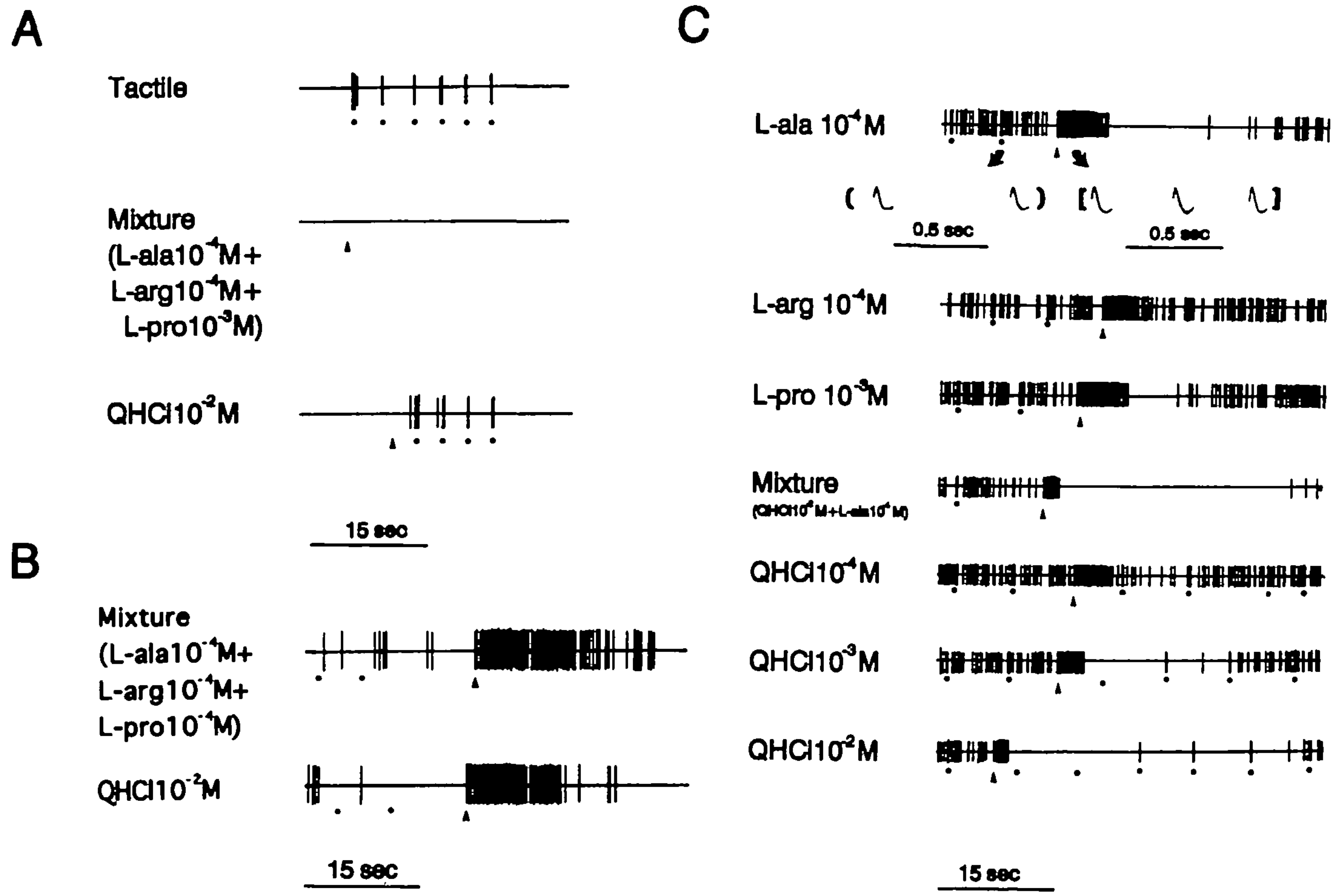

Figure 1 Representative responses from single fibers of the facial/trigeminal complex in the channel catfish Responses of tactile-only **(A)**, taste-only **(B)** and bimodal (taste/tactile) **(C)** fibers are illustrated. Tactile-only fibers (A) were non-responsive to amino acids and quinine, but responded phasically to mechanical stimulation. Taste-only fibers (B) responded to chemical (amino acid), but not to mechanical stimulation. Black dots below the spike sequences in (A–C) represent punctate mechanical stimulation of the receptive field with the fine tip of a glass probe. Bimodal fibers (C) responded to taste (amino acid and quinine) and tactile stimulation. Arrowheads in (A) indicate the application of taste stimulus; arrowheads in (B, C) indicate the beginning of the taste responses. Arrows in (C) show the similarity in the waveforms of the action potentials in this fiber evoked by tactile and taste stimulation. Transient suppression by quinine of the tactile responses of bimodal [(C), last three traces], but not tactile-only [(A), trace 3] fibers occurred QHCL, quinine hydrochloride.

had been tapered by heating and pulling with a standard microelectrode puller (fire-polished tip diameter <1 mm). For the application of the tactile stimulus, the glass probe touched the surface of the maxillary barbel intermittently from its tip to base. A tactile response was said to occur if a phasic burst of action potentials occurred coincident with each tactile stimulation. If tactile activity did not result from punctate stimulation, gliding movements of the probe were directed along the axis of the barbel. If phasic bursts of action potentials occurred coincident with the gliding movement of the probe, a tactile response was indicated to have occurred. Whether mechanical responses occurred or not, a gustatory stimulus consisting of a mixture of 10^{-4} M L-alanine, 10^{-4} M L-arginine and 10^{-3} M L-proline, the three amino acids that best activate different facial taste fibers in the channel catfish (Kohbara *et al.*, 1992), was presented. For some fibers, each of the amino acids in the trinary mixture and 10^{-4}–10^{-2} M quinine-HCl were tested individually. A taste response was said to occur if a phasic burst of action potentials occurred in response to the chemical stimulus onset. The phasic taste response was always more prolonged than the response to the tactile stimulus (see Figure 1) and was delayed by a few seconds from the application of the chemical stimuli to the stimulus delivery system. A few seconds was required for the chemical stimulus, once injected into the continuous flow of water

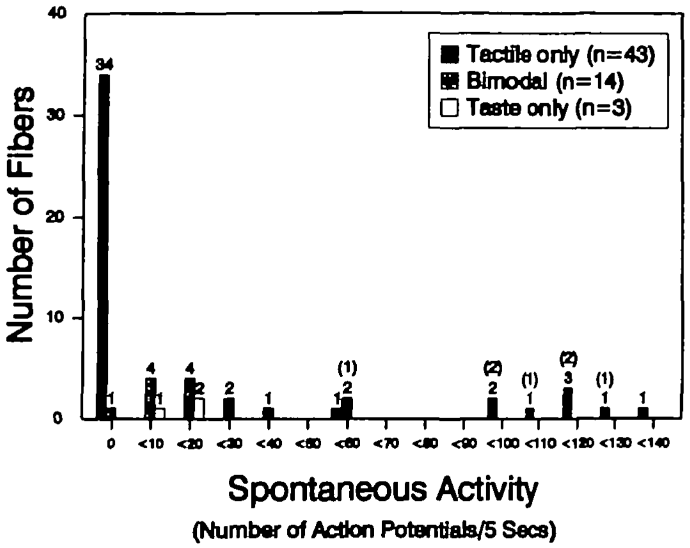

Figure 2 Distribution of spontaneous activities of single fibers dissected from the facial/trigeminal complex innervating the maxillary barbel in the channel catfish Values along the abscissa preceded by the < symbol include a range of action potentials from the previous lower abscissal value to less than the particular value indicated (e.g <60 indicates the number of fibers whose spontaneous activity ranged between 50 and 59 action potentials/5 s). Numbers above each bar indicate the number of fibers in each specified category Bracketed numbers above bars indicate those fibers that were suppressed by tactile stimulation None of the bimodal or taste only fibers was suppressed by the amino acid taste stimuli.

directed to the maxillary barbel, to contact the taste receptive field of the recorded facial taste fiber. Since the spontaneous activity of the taste-responsive fibers was relatively low (Figure 2), the taste response was clearly evident (Figure 1B, C).

A total of 60 single unitary responses was identified from the maxillary branch of the facial/trigeminal nerve complex, whose receptive fields were restricted to the maxillary barbel. Forty-three of 60 single fibers responded only to tactile stimulation (Figure 1A). Of these 43 fibers, 34 were not spontaneously active and were excited by the mechanical stimulus (Figure 1A). Eight of the nine remaining fibers had a relatively high (>90 spikes/5 s) spontaneous rate and six of these were suppressed by tactile stimulation (Figure 2). The three fibers that responded to the amino acid stimuli, but not the tactile stimulation, had low (<20 action potentials/5 s) spontaneous activity and were excitatory (Figure 1B, top trace). The remaining 14 (23%) fibers responded bimodally to both amino acid and tactile stimulation (Figure 1C). With only two exceptions, the spontaneous activity of these bimodal fibers did not overlap with that for the tactile-only fibers (Figure 2). Another physiological difference between tactile-only fibers and fibers responsive to chemical stimuli was conduction velocity, where the 3.4 m/s conduction velocity of bimodal (taste/tactile) and taste-only chorda tympani fibers of the rat did not overlap with the faster 10 m/s conduction velocity of tactile-only fibers (Matsuo *et al.*, 1995). With the single exception in the present report of one bimodal fiber that responded with suppression to tactile stimulation, all bimodal fibers responded well to both amino acid and mechanical stimulation (Figure 2). The 23% of bimodal single fibers observed in the channel catfish is two (Kasahara and Kawamura, 1975) to four (Matsuo *et al.*, 1995) times that reported for the rat.

It is most probable that the single fibers in the present report that responded with high sensitivity to amino acids (i.e. both the taste-only and the bimodal units) were facial fibers that innervated some of the numerous taste buds present on the maxillary barbel, whereas the 43 tactile-only fibers could have been either facial or trigeminal fibers or a combination of these types. Although it was reported that the trigeminal nerve of both cod (*Gradus morchua maris-albi*) and carp (*Cyprinus carpio*) responds to amino acids, the recordings (Belousova *et al.*, 1983) were obtained from the buccalis nerve, which is a mixed nerve containing facial, trigeminal and anterior lateral-line components (Herrick, 1899, 1901; Luiten, 1975). Thus, the recordings to amino acids which were applied to the olfactory organ and to skin around the nasal sac could have stimulated taste buds, neuromasts (Kawamura and Tamura, 1980a,b; Kawamura and Yamashita, 1981), or even solitary chemosensory cells (SCCs) (Whitear, 1992). The present responses to amino acids were evidently obtained from facial taste fibers innervating taste buds, as neither neuromasts nor SCCs are known to occur on the barbels of the channel catfish.

Although the source of the tactile responsiveness of the bimodal fibers was not identified, its origin(s) could be within the taste bud (intragemmal) and/or external to the taste bud (extragemmal). Dopaminergic basal cells, which lie within the teleost taste bud at its base and synapse with taste cells and gustatory fibers, may function as mechano- sensory interneurons (Reutter, 1978; Toyoshima *et al.*, 1984). Extragemmal free nerve endings may also be a source of the tactile responsiveness of the bimodal facial fibers.

In the classification of maxillary barbel fibers into taste-only and tactile-only categories, the assumption is the fibers that failed to respond to the trinary mixture of the L-isomers of alanine, arginine and proline were not responsive to other amino acids (Kohbara *et al.*, 1992) or to other non-amino acid taste stimuli. There is evidence that a small proportion (<16%) of facial taste fibers in the channel catfish is poorly sensitive to amino acids, but does respond to specific nucleotides (Kohbara *et al.*, 1992). If some of these fibers were included in the fibers tested here, then the percentage of both bimodal and taste-responsive fibers may have slightly increased. With the exception of the few nucleotide sensitive fibers, a taste fiber that does not respond to high (0.1, 1.0 mM) concentrations of L-alanine, L-arginine and L-proline is unresponsive to other stimuli tested, including other amino acids (Kohbara *et al.*, 1992). Whether taste fibers that were indicated as being tactile-only would have responded to some unnatural stimulus, such as hydrochloric acid, was not considered relevant to this study.

Stimulation with quinine (hydrochloride) in the present study provided additional support for differences between the two types of fibers that responded to mechanical stimulation. Concentrations of 10 mM quinine transiently suppressed the mechanosensitivity of seven of the nine bimodal fibers tested (Figure 1C); this effect was also sometimes observed with 1 mM quinine (Figure 1C). Of the 38 tactile-only fibers tested, none was affected by quinine application (Figures 1A and 2).

The present report suggests that the high percentage of bimodal taste/tactile units observed at the level of the primary gustatory nucleus in vertebrates may not be the result solely of the convergence of primarily gustatory and tactile fibers respectively, but may in some species, such as the channel catfish, be due to a significant input of bimodal facial fibers.

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