The Journal of Experimental Biology 211, 2786-2791
Published by The Company of Biologists 2008
doi:10.1242/jeb.018648

Bile salts are effective taste stimuli in channel catfish

S. H. Rolen* and J. Caprio

Department of Biological Sciences, Louisiana State University, Life Sciences Building Room 107 Baton Rouge, LA 70803, USA

*Author for correspondence (e-mail: srolen1@lsu.edu)

Accepted 9 June 2008

SUMMARY

Bile salts are known olfactory stimuli for teleosts, but only a single report has indicated that the taste system of a fish was sensitive to this class of stimuli. Here, gustatory responses of the channel catfish, *Ictalurus punctatus*, to four bile salts that included taurine-, glycine- and non-conjugated compounds along with three stimulatory amino acids as a comparison were investigated using extracellular electrophysiological techniques. Integrated multiunit responses were obtained from the branch of the facial nerve innervating taste buds on the maxillary barbel. Bile salts were shown to be highly effective facial taste stimuli, with estimated electrophysiological thresholds for three of the four tested bile salts of approximately 10^{-11} mol l^{-1} to 10^{-10} mol l^{-1}, slightly lower by 1–2 log units than those to amino acids in the same species. Although the sensitivity of the facial taste system of the channel catfish to bile salts is high, the relative magnitude of the response to suprathreshold concentrations of bile salts was significantly less than that to amino acids. Multiunit cross-adaptation experiments indicate that bile salts and amino acids bind to relatively independent receptor sites; however, nerve-twig data and single-fiber recordings suggest that both independent and shared neural pathways exist for the transmission of bile salt and amino acid information to the primary gustatory nucleus of the medulla.

Key words: taste, bile salts, catfish, amino acids, electrophysiology.

INTRODUCTION

Chemosensory systems of fishes detect and discriminate biologically relevant environmental cues conveying information pertaining to conspecifics, spawning habitats and food sources (Sorensen and Caprio, 1998). These chemosensory systems are uniquely different from those of terrestrial vertebrates in that both olfactory and gustatory stimuli are dissolved in an aqueous solution. Specifically for teleosts, a variety of water-soluble molecules (amino acids, bile salts, nucleotides, polyamines and sex pheromones) was previously identified as potent chemosensory stimuli (Michel et al., 2003; Rolen et al., 2003; Sorensen and Caprio, 1998; Caprio and Derby, 2008). To understand better how chemosensory information for a given class of stimuli is processed by teleosts, investigations of both the gustatory and olfactory systems are required.

Over the past 30 years, the detection and processing of amino acid stimuli by the chemosensory systems of teleosts have been well studied (Sorensen and Caprio, 1998; Caprio and Derby, 2008); however, knowledge of the response specificity of the olfactory and gustatory systems of the same species to these stimuli is sparse, especially considering the large number of teleost species that currently exist (Hara, 1975; Caprio, 1978; Goh and Tamura, 1980; Marui et al., 1983; Hara et al., 1999; Yamashita et al., 2006). For these studies, amino acids were shown to be potent stimuli for both chemosensory systems of specific species, but some major differences were indicated with respect to the relative stimulatory effectiveness of the stimuli.

Bile salts are another class of biologically relevant olfactory stimuli in fishes (Døving et al., 1980; Li et al., 1995; Friedrich and Korsching, 1998; Zhang et al., 2001; Nikonov and Caprio, 2001; Rolen and Caprio, 2007). These compounds are biliary steroids derived from cholesterol, synthesized by the liver, stored in the gall bladder and released into the intestinal lumen to emulsify fats and aid in the absorption of lipids and fat-soluble vitamins (Haslewood, 1967). Although most bile salts are reabsorbed by the enterohepatic system, in fishes some are released into the water column in feces and urine and can function as chemosensory cues (Polkinghorne et al., 2001; Fine and Sorensen, 2005; Zhang et al., 2001).

Previously, all chemosensory investigations but one (Yamashita et al., 2006), which investigated the taste system of the rainbow trout, studied the detection of bile salts by the olfactory system. The present study examines whether bile salts are effective facial taste stimuli in the channel catfish. The results indicate that electrophysiological thresholds are in the low-nanomolar range and that bile salts are processed by facial neural pathways both independent from those processing amino acids and pathways conveying both types of taste information.

MATERIALS AND METHODS

Experimental animals

Channel catfish (*Ictalurus punctatus* Rafinesque), 15–22 cm in length, were obtained from indoor recirculating tanks at the Louisiana State University (LSU, LA, USA) Aquaculture Facility. Fish were held in the LSU Animal Care Facility in a 300 l aquarium filled with charcoal-filtered tap water (CFTW) and maintained on a 12 h:12 h light:dark regime for up to 2 weeks. The temperature of the aquarium water was held at 31°C.

Animal preparation

The procedures outlined are in accordance with a protocol approved by the Institutional Animal Care and Use Committee (LSU School of Veterinary Medicine).

Each catfish was immobilized with an initial intramuscular injection of Flaxedil (gallamine triethiodide, 0.03 mg/100 g body mass). Subsequent injections of Flaxedil were provided as needed during experimentation by means of a hypodermic needle embedded in the flank musculature. The catfish was wrapped in wet tissue

paper and secured to a wax block in a customized Plexiglas chamber. The gills were irrigated by a constant flow of CFTW containing the general anesthetic MS-222 (ethyl-*m*-aminobenzoate methane sulfonic acid; initial concentration, 50 mg/l; Sigma Chemical, St Louis, MO, USA) for the duration of the experiment. The local anesthetic tetracaine (3% mass/vol.) was applied locally to the skin before surgical procedures to expose by deocculation a portion of the mandibular branch of the facial–trigeminal nerve complex that innervates taste buds on the caudal portion of the maxillary barbel. A mandibular branch of the facial–trigeminal complex that innervates the caudal maxillary barbel was selected for recording as it is separate from the other large nerve branches of the complex that innervate the rostral portion of the head. Procedures for surgical exposure have been described previously (Caprio, 1995). Following the surgical procedures, the connective tissue encasing the nerve branch was removed, and the nerve was cut at its most visible caudal point in the orbit. Depending upon the preparation, recordings were obtained from either the whole nerve, nerve twigs or from a few fibers. Neural activity was recorded with a tungsten hook electrode, AC amplified (Grass Instruments P511, Quincy, MA, USA; bandpass 30–3000 Hz), integrated (only for whole-nerve and nerve-twig preparations), monitored aurally, displayed on an oscilloscope and a DC chart recorder and stored on the audio channel of a high-fidelity VCR.

Chemical stimuli

The test stimuli included L-amino acids (alanine [Ala], arginine [Arg] and proline [Pro]) and bile salts (sodium salts of chenodeoxycholic acid [CDC], glycochenodeoxycholic acid [GCDC], taurochenodeoxycholic acid [TCDC] and taurocholic acid [TCA]). Alanine, arginine and proline were shown previously to be highly potent taste stimuli for the channel catfish (Caprio, 1978; Kohbara et al., 1992). The four bile salts tested in this study were selected to include two produced by the channel catfish [TCDC and TCA (Kellogg, 1975)] and two bile salts (GCDC and CDC) that have a close structural resemblance to TCDC and TCA (Fig. 1). Previous investigators (Døving et al., 1980; Goh and Tamura, 1980; Jones and Hara, 1985; Hellstrøm and Døving, 1986; Friedrich and Korsching, 1998; Nikonov and Caprio, 2001; Zhang et al., 2001; Rolen et al., 2003; Rolen and Caprio, 2007) commonly utilized one or more of these bile salts in their studies. The molecular features of TCDC, GCDC and CDC differ only by the molecular moiety conjugated to carbon 24 (C24). TCA contains a hydroxyl group at C12, whereas this molecular feature is a hydrogen atom in the other three tested bile salts. The variation in the conjugating group at C24 among these bile salts affords the ability to test three different classes of bile salts (taurine-, glycine- and non-conjugated). All four bile salts tested were 3α, 5β, 7α and 12α isomers. All chemical stimulants were purchased from Sigma Chemical and were of the highest purity available (97%–99%). Stock solutions of amino acids and bile salts were prepared weekly using CFTW and were refrigerated when not in use. Test solutions were diluted daily from stock solutions to experimental concentrations with CFTW and were tested at room temperature, the same as that of the water flow to the maxillary barbel.

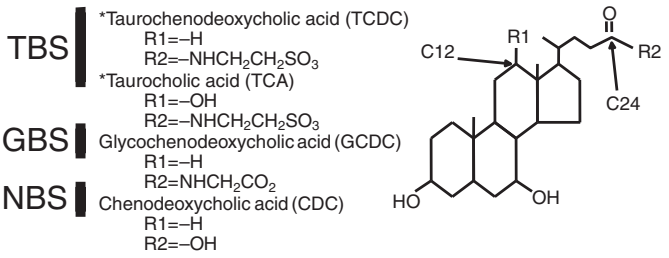

Fig. 1. The molecular structures of the bile salts tested. Molecular features, designated by R1 and R2, of each bile salt tested vary at carbon positions C12 of the steroid backbone and C24 of the side-chain, respectively. The stimuli included different classes of bile salts based on the specific molecular feature (R2) attached to C24 [glycine-conjugated (GBS), taurine-conjugated (TBS), non-conjugated (NBS)]. All of the bile salts are 3α, 5β, 7α and 12α isomers. An asterisk indicates those bile salts produced by the channel catfish (Kellogg, 1975).

Stimulus delivery

Stimulus delivery was by means of a 'gravity-feed' system, which has been described previously (Sveinson and Hara, 2000). The maxillary barbel was inserted into a glass sleeve and continuously bathed in CFTW (flow rate, 8–10 ml min^{-1}) not containing MS-222, or during cross-adaptation experiments (see below), continuously bathed by the adapting solution. Briefly, stimulus solutions and the CFTW used to bathe the maxillary barbel were delivered through separate Teflon tubes (diameter 0.8 mm) to a common tube that extended 46 cm to the maxillary barbel. A foot switch connected to an electronic timer (Model 645, GraLab Instruments Division, Dimco-Gray Corporation, Centerville, OH, USA) triggered a pneumatic actuator valve to introduce the stimulus for applications of duration 2 s. With the sole exception of when a stimulus was added, CFTW alone continuously perfused the maxillary barbel to: (i) prevent desiccation, (ii) facilitate stimulus delivery, (iii) avoid the introduction of mechanical artifacts associated with stimulus presentation and (iv) rinse the glass sleeve containing the maxillary barbel clear of any residual stimuli for a minimum of 2 min between stimulus applications.

Cross-adaptation experiments

Electrophysiological cross-adaptation experiments to determine the relative independence of the neural pathways for the stimuli consisted of three stages: (i) Pre-adaptation: CFTW continuously bathed the left maxillary barbel for a minimum of 5 min before stimulus applications. Bile salts and amino acids were tested at 10^{-5} mol l^{-1} and 10^{-6} mol l^{-1}, respectively. CFTW served as the control during pre-adaptation. (ii) Adaptation: the adapting solution continuously bathed the maxillary barbel. All stimuli tested during adaptation were dissolved in the adapting solution. The adapting solution served as the control and was tested immediately before each test stimulus. If responses to the test stimuli were suppressed to the control level (complete adaptation), these test stimuli were considered to share the same neural pathways as the adapting stimulus. If the responses to test stimuli were significantly greater than the control level, these test stimuli were considered to have at least partially independent receptor sites and neural pathways from the adapting stimulus. (iii) Post-adaptation, CFTW continuously bathed the maxillary barbel for 5 min before stimulus application. Stimuli and controls were identical to those described during pre-adaptation.

RESULTS

Characteristics of the integrated taste responses to bile salts

Initially, integrated multiunit responses to bile salts were recorded from the entire branch of the facial–trigeminal nerve complex innervating the caudal portion of the maxillary barbel; however, it is the facial nerve components from which taste activity is recorded. These recordings permitted an evaluation of the stimulatory

effectiveness of bile salts relative to that for amino acids – the more well-established tastants for channel catfish (Caprio, 1978; Kohbara et al., 1992). Only prominent phasic responses were evident for both classes of stimuli (Fig. 2); the neural activity increased as the stimuli contacted the maxillary barbel and returned to pre-stimulus levels without any obvious tonic level of activity. A stimulus duration of 2 s was chosen for the remaining experiments. Dose–response data indicate that thresholds for the more effective bile salts tested were as low as 10^{-11}–10^{-10} mol l^{-1}, with the magnitude of the integrated response generally increasing with stimulus concentration up to approximately micromolar concentrations (Figs 3 and 4). The four tested bile salts (TCDC, TCA, CDC and GCDC, each at 10^{-6} mol l^{-1}) evoked mean responses ≤50% of that evoked by the standard, 10^{-6} mol l^{-1} L-alanine (Fig. 4).

Cross-adaptation experiments: evidence for the relative independence of receptor sites for amino acids and bile salts

During continuous application of 10^{-5} mol l^{-1} TCDC to the maxillary barbel, the integrated responses to GCDC and CDC, each at 10^{-5} mol l^{-1}, were reduced to 12.8±11.1% and 11.1±10%, respectively (i.e. control level; one-way ANOVA; Tukey's *post hoc* test, $P>0.05$) of their unadapted responses, whereas responses to L-amino acids were unaffected (Fig. 5A, Fig. 6A). During continuous application of 10^{-6} mol l^{-1} L-amino acids (Ala, Arg and Pro), the integrated multiunit responses to 10^{-5} mol l^{-1} bile salts were reduced to only ~69.7%–81.9% of their unadapted responses (Fig. 5B, Fig. 6B).

Nerve-twig and unit data: evidence for both distinct and shared neural pathways in the facial nerve

To test for the possibility of independent neural pathways for bile salts and amino acids, the nerve innervating the caudal portion of the maxillary barbel was carefully teased into multiple bundles. We reasoned that if separate neural pathways for the transmission of bile salt and amino acid information existed within this nerve and the relative number of fibers most responsive to these stimuli differed across the bundles then the ratio of taste responses to bile salts and amino acids would also differ across the tested nerve bundles. A total of 18 nerve bundles from three channel catfish were tested with a mixture of 10^{-5} mol l^{-1} bile salts (TCDC, TCA, GCDC and CDC) and a mixture of 10^{-6} mol l^{-1} L-amino acids (Ala, Arg and Pro). As hypothesized, the integrated response to bile salts varied in comparison to that for amino acids across the 18 nerve twigs tested (Fig. 7). The integrated taste responses elicited by the bile-salt mixture were averaged and expressed as a percentage of the averaged integrated taste response to the amino acid mixture obtained from each nerve twig. The magnitude of the integrated response recorded to the bile-salt mixture ranged from 0% to >100% of the response to the mixture of 10^{-6} mol l^{-1} amino acids (Table 1). The response to amino acids and not to bile salts for at least a portion of the twig data (Fig. 7A) confirms the independence of both receptor sites and at least a portion of the neural pathways for the tested stimuli.

A few (N=11) single fibers were also isolated and tested with bile salts and amino acids to investigate further the specificities of the neural pathways for these stimuli. From a total of 11 single fibers obtained, two were excited solely by bile salts (Fig. 8A), five solely by amino acids (Fig. 8B), and four fibers were excited by both types of stimuli (Fig. 8C).

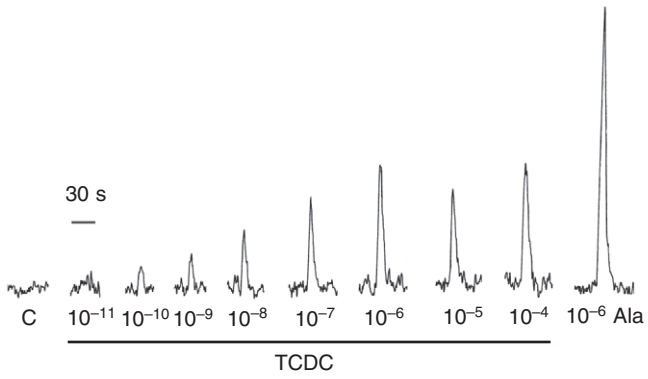

Fig. 3. Integrated taste responses to 10^{-11} to 10^{-4} mol l^{-1} TCDC recorded from the entire branch of the facial–trigeminal complex that innervates the caudal portion of the maxillary barbel. Responses to CFTW (charcoal-filtered tap water; C) control and 10^{-6} mol l^{-1} L-alanine are shown to allow comparisons.

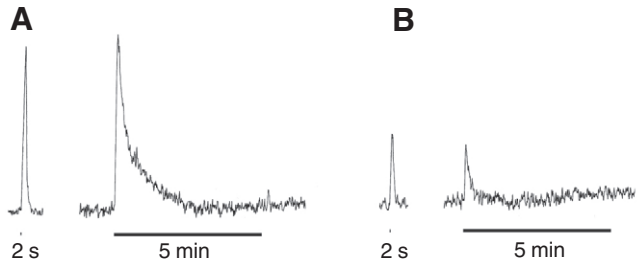

Fig. 2. Integrated whole-nerve taste responses to (A) 10^{-6} mol l^{-1} L-alanine and (B) 10^{-5} mol l^{-1} TCDC. Each compound was tested at two stimulus durations, 2 s and 5 min. Note that each compound evoked only phasic responses regardless of the stimulus duration.

DISCUSSION

Response characteristics to bile salts

Electrophysiological responses to bile salts were recorded from the facial nerve fibers that innervate taste buds and reside within the branch of the facial–trigeminal complex that innervates the caudal

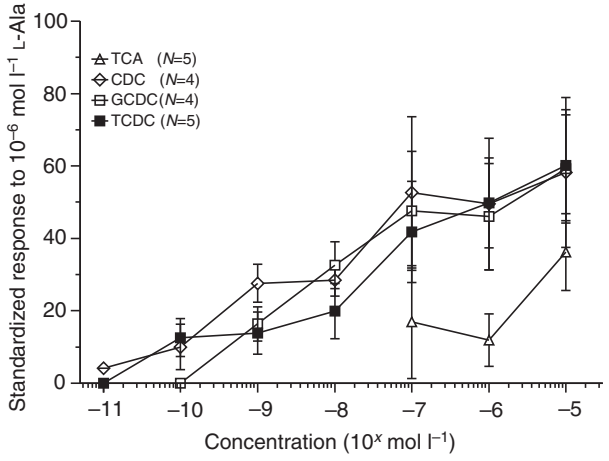

Fig. 4. Dose–response plots of integrated taste responses to bile salts standardized to the response to 10^{-6} mol l^{-1} L-alanine. The number of fish tested (N) is provided in the key. The averaged control magnitude value was subtracted from the averaged stimulus magnitude response at each concentration. Data points and error bars, means ±s.e.m.

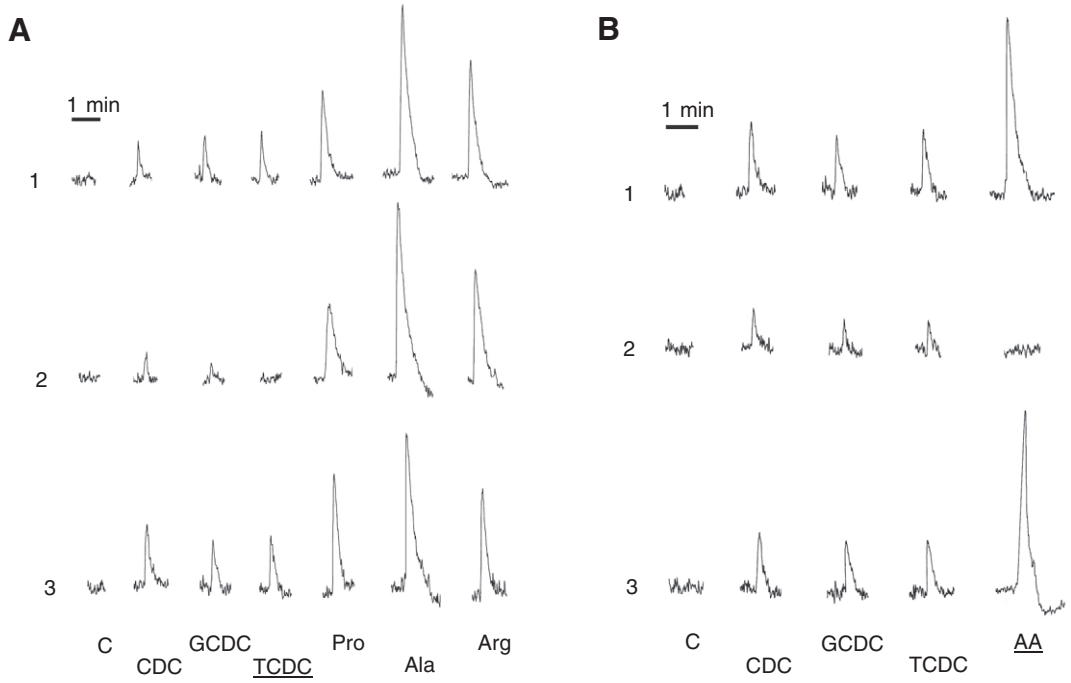

Fig. 5. Representative cross-adaptation experiments illustrating the integrated taste activity recorded (1) before, (2) during and (3) after adaptation to (A) 10^{-5} mol l^{-1} TCDC and (B) a mixture of 10^{-6} mol l^{-1} L-amino acids (Ala, Arg and Pro; AA). The adapting solution is underlined.

maxillary barbel. Integrated multiunit activity in response to bile salts was fast-adapting, displaying only a phasic response, irrespective of stimulus duration, that is similar to that obtained for amino acids in both this report and as described previously (Caprio, 1978). However, a phasic-only response to bile salts in the channel catfish is in direct contrast to responses to bile salts in rainbow trout, where both phasic and tonic components of the gustatory response were clearly evident (Yamashita et al., 2006).

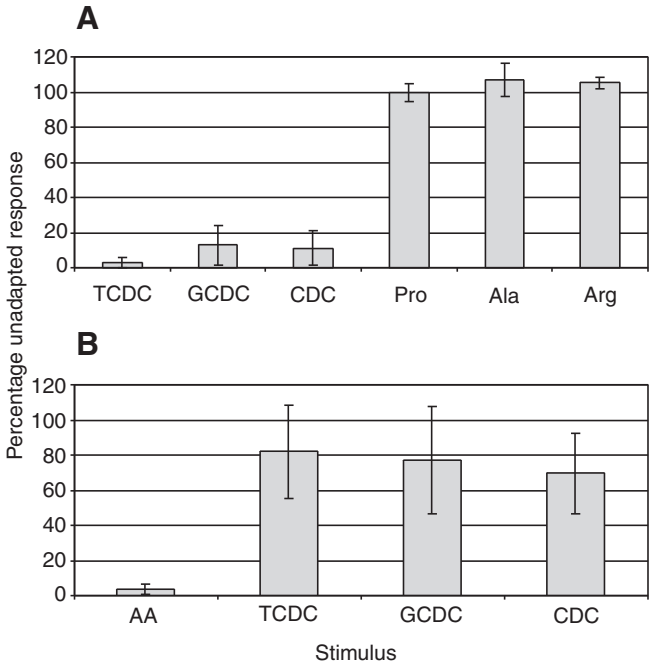

Fig. 6. Results of cross-adaptation experiments. (A) Adaptation to 10^{-5} mol l^{-1} TCDC; (B) adaptation to a mixture of 10^{-6} mol l^{-1} amino acids (Ala, Arg and Pro). Bars indicate the percentage of the unadapted response (means ± s.d.). Responses significantly greater than control responses: (A) Pro, Ala and Arg; (B) TCDC, GCDC and CDC (one-way ANOVA; Tukey's *post hoc* test, $P<0.05$). $N=3$ fish tested.

Dose–response properties

Dose–response plots of the integrated multiunit taste activity demonstrate that taste thresholds to three of the four bile salts tested were ~10^{-11} mol l^{-1} to 10^{-10} mol l^{-1}. Of the two bile salts tested (TCDC and TCA) that are produced by channel catfish, TCDC was one of the more effective taste stimuli. TCA was a relatively poor stimulus compared both with TCDC and with the two additional bile salts tested, CDC and GCDC, that are not produced by channel catfish. The data indicate that thresholds to bile salts are lower than those for amino acids in the facial taste system in the same species (Kohbara et al., 1992) and are 1–2 log units higher than those recorded for TCA in the rainbow trout (Yamashita et al., 2006). Although the thresholds to bile salts were lower than those to amino acids in the facial taste system, amino acids elicited greater integrated response magnitudes at equivalent stimulus concentrations. The magnitude of the response to the standard, 10^{-6} mol l^{-1} L-alanine, was typically twice that to the more potent bile salts tested. In all recordings from the entire nerve branch innervating the caudal maxillary barbel, the responses to 10^{-6} mol l^{-1} bile salts never exceeded ~50% of the response to 10^{-6} mol l^{-1} L-alanine.

Currently, there are no published reports of comparable data investigating the stimulatory effectiveness of bile salts to olfactory receptor neurons in channel catfish. However, single olfactory bulb neurons in this species responded excitedly to bile salts at concentrations between 10^{-7} mol l^{-1} and 10^{-6} mol l^{-1} (Rolen and Caprio, 2007). Thus, the gustatory system of channel catfish is ~1000–10,000 times more sensitive to this class of molecules than its olfactory counterpart. By comparison, olfactory and gustatory thresholds to bile acids in salmonids might not be so disparate as

Table 1. The number of nerve twigs recorded whose response magnitude to bile salts is expressed as a percentage of the response magnitude to the mixture of 10^{-6} mol l^{-1} amino acids

Response magnitude	0% to <20%	20% to <40%	40% to <60%	60% to <80%	80% to 100%	>100%
No. of nerve twigs	7	5	3	1	1	1

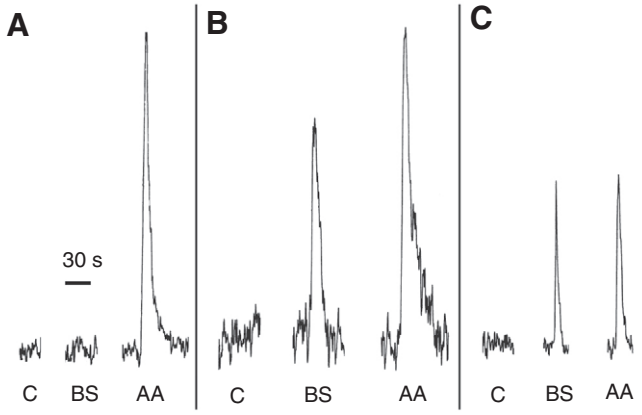

Fig. 7. Integrated multiunit taste recordings from three separate facial nerve twigs (A–C) innervating the maxillary barbel in a single fish showing the variability of the magnitude of the integrated responses to bile salts with respect to amino acids. (A) Nerve twig lacking a bile-salt response but showing a large-magnitude amino acid response. (B) Nerve twig with a significant bile-salt response and an even-greater-magnitude amino acid response. (C) Nerve twig responding approximately equally to the bile salt and amino acid mixtures. C, CFTW control; BS, a mixture of 10^{-5} mol l^{-1} TCDC, TCA, GCDC and CDC; AA, a mixture of 10^{-6} mol l^{-1} L-alanine, L-arginine and L-proline.

olfactory thresholds for the more stimulatory bile acids in salmonids estimated from integrated olfactory bulb waves ranged between 10^{-9} mol l^{-1} and 10^{-11} mol l^{-1} (Døving et al., 1980), whereas the taste threshold to taurocholic acid in rainbow trout was 10^{-12} mol l^{-1} (Yamashita et al., 2006).

Taste receptor sites for bile salts

Cross-adaptation experiments in the present study indicated the relative independence of taste receptor sites for bile salts and amino acids, which is similar to that reported for rainbow trout (Yamashita et al., 2006). The cross-adaptation data also suggest that the three bile salts tested individually bind to the same receptor as adaptation with TCDC eliminated to control level the responses to CDC and GCDC. These results are also similar to those observed in the rainbow trout (Yamashita et al., 2006). However, as single olfactory bulb neurons in the channel catfish could discriminate between different molecular features of specific bile salts (Rolen and Caprio, 2007), it is possible that relatively independent taste receptor sites exist for other untested biliary steroids.

Bile salt and amino acid taste information is processed by both independent and shared neural pathways

The present nerve-twig and single-fiber data suggest that both independent and shared neural taste pathways exist for bile salts in the channel catfish. Small teased branches of the nerve innervating the caudal maxillary barbel were responsive to amino acids and not bile salts. Furthermore, single-fiber data confirmed that a portion of the facial nerve neural pathways conveying bile-salt taste information is separate from those pathways conveying amino acid taste information, as evidenced by single fibers responsive only to the bile salt or to the amino acid mixtures. However, single fibers responding excitedly to both the bile salt and amino acid mixtures were also observed, suggesting that some degree of overlap also occurs. Single taste fibers responding to structurally different classes of tastants have been demonstrated previously for *Seriola quinqueradiata*, where single palatine taste fibers responded to both amino acid and nucleotide stimuli (Zeng and Hidaka, 1990).

Behavioral implications

The present study, combined with data from a previous investigation (Yamashita et al., 2006), indicates that channel catfish and rainbow trout possess gustatory systems capable of detecting bile salts. Currently, there are no published investigations citing specific

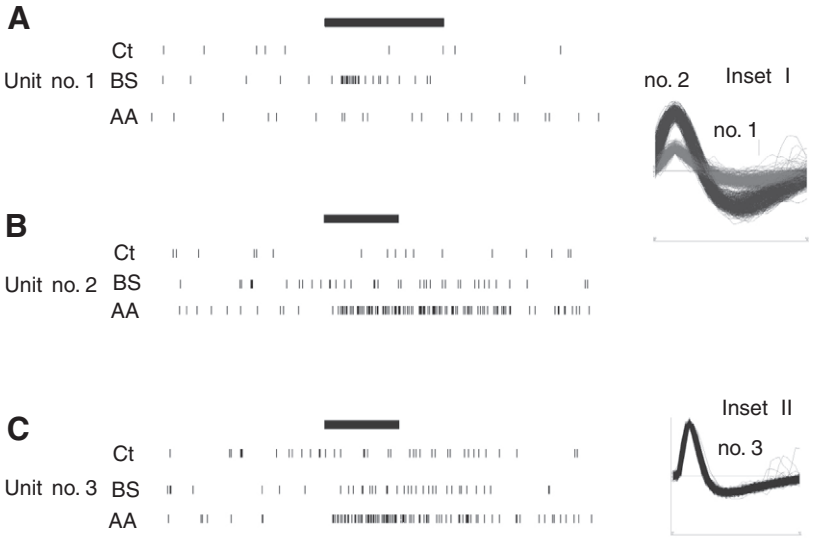

Fig. 8. Responses of single facial taste fibers. (A) A single fiber responding only to the bile salt (BS) mixture (10^{-5} mol l^{-1} TCDC, TCA, GCDC and CDC); (B) a single fiber responding only to the amino acid (AA) mixture (10^{-6} mol l^{-1} Ala, Arg and Pro); (C) a single fiber responding to both stimulus mixtures. A and B were recorded simultaneously from different fibers in the same preparation, whereas unit no. 3 was recorded from a different animal. Clusters of individual spike trains are shown in insets I and II to indicate that the action potentials recorded in A–C were evoked by single fibers. Ct, CFTW. Horizontal bars indicate 2 s stimulus applications.

behaviors resulting from gustatory detection of bile salts in either fish. To date, the olfactory detection of bile salts and its role in sea lamprey migration is the most well-documented case of a direct effect of biliary steroids on the behavior of a fish. It is hypothesized that olfactory recognition and discrimination of specific sea lamprey bile salts are key for successful migration of adult sea lampreys to suitable spawning habitats. Sexually mature sea lampreys innately recognize a mixture of species-specific bile salts (Li et al., 1995; Li et al., 2002; Fine et al., 2004; Sorensen et al., 2005) and select for streams containing populations of sea lamprey larvae, indicative of suitable spawning grounds (Bjerselius et al., 2000; Polkinghorne et al., 2001; Vrieze and Sorensen, 2001; Fine and Sorensen, 2005). Previous investigations demonstrated that freshwater eels (Sola and Tosi, 1993), Artic char (Jones and Hara, 1985) and cod (Hellstrøm and Døving, 1986) respond to synthetic bile salts, with activities classified as orientation and snapping. Furthermore, Hellstrøm and Døving (Hellstrøm and Døving, 1986) showed that TCA was detected in the absence of a functioning olfactory system. Future behavioral investigations are needed to determine to role of gustation in the detection of bile salts for both channel catfish and other species.

This work was supported by the National Science Foundation Grant IBN-0314970.

REFERENCES

Bjerselius, R., Li, W., Teeter, J. H., Johnsen, P. B., Maniak, P. J., Grant, G. C., Polkinghorne, C. N. and Sorensen, P. W. (2000). Direct behavioral evidence that unique bile acids released by larval sea lamprey (*Petromyzon marinus*) function as a migratory pheromone. *Can. J. Fish. Aquat. Sci.* **57**, 557-569.

Caprio, J. (1978). Olfaction and taste in the channel catfish: an electrophysiological study of the responses to amino acids and derivatives. *J. Comp. Physiol. A* **123**, 357-371.

Caprio, J. (1995). *In vivo* olfactory and taste recordings in fish. In *Experimental Neuron Biology of Taste and Olfaction (Current Techniques and Protocols)* (ed. A. I. Spielman and J. G. Brand), pp. 251-261. Boca Raton: CRC.

Caprio, J. and Derby, C. D. (2008). Aquatic animal models in the study of chemoreception. In *The Senses: A Comprehensive Reference* (ed.A. I. Basbaum, A. Kaneko, G. M. Shepherd and G. Westheimer), pp. 97-134. San Diego: Academic Press.

Døving, K. B., Selset, R. and Thommsen, G. (1980). Olfactory sensitivity to bile salts in salmonid fishes. *Acta Physiol. Scand.* **108**, 123-131.

Fine, J. M. and Sorensen, P. W. (2005). Biologically relevant concentrations of petromyzonol sulfate, a component of the sea lamprey migratory pheromone, measured in stream water. *J. Chem. Ecol.* **31**, 2205-2210.

Fine, J. M., Vrieze, L. A. and Sorensen, P. W. (2004). Evidence that petromyzontid lampreys employ a common migratory pheromone that is partially comprised of bile acids. *J. Chem. Ecol.* **30**, 2091-2110.

Friedrich, R. W. and Korsching, S. I. (1998). Chemtopic, combinatorial, and noncombinatorial odorant representations in the olfactory bulb revealed using a voltage-sensitive axon tracer. *J. Neurosci.* **18**, 9977-9988.

Goh, Y. and Tamura, T. (1980). Olfactory and gustatory responses to amino acids in two marine teleosts: Red sea bream and mullet. *Comp Biochem Physiol C* **66**, 217-224.

Hara, T. J. (1975). Olfaction in fish. *Prog. Neurobiol.* **5**, 271-335.

Hara, T. J., Carolsfeld, J. and Kitamura, S. (1999). The variability of the gustatory sensibility in salmonids, with special reference to strain differences in rainbow trout, *Oncorhynchus mykiss*. *Can. J. Fish. Aquat. Sci.* **56**, 13-24.

Haslewood, G. A. D. (1967). *Bile Salts*. Suffolk: Chaucer.

Hellstrøm, T. and Døving, K. B. (1986). Chemoreception of taurocholate in anosmic and sham-operated cod. *Gadus morhua*. *Behav. Brain Res.* **21**, 155-162.

Jones, K. A. and Hara, T. J. (1985). Behavioral responses of fishes to chemical cues: Results from a new bioassay. *J. Fish Biol.* **27**, 495-504.

Kellogg, T. F. (1975). The biliary bile acids of the channel catfish, *Ictalurus punctatus*, and the blue catfish, *Ictalurus furcatus*. *Comp. Biochem. Physiol., B* **50**, 109-111.

Kohbara, J., Michel, W. and Caprio, J. (1992). Responses of single facial taste fibers in the channel catfish, *Ictalurus puntatus*, to amino acids. *J. Neurophysiol.* **68**, 1012-1026.

Li, W., Sorensen, P. W. and Gallaher, D. D. (1995). The olfactory system of migratory adult sea lamprey (*Petromyzon marinus*) is specifically and acutely sensitive to unique bile salts released by conspecifics larvae. *J. Gen. Physiol.* **105**, 569-587.

Li, W., Scott, A. P., Siefkas, M. J., Yan, H., Liu, Q., Yun, S. and Gage, D. A. (2002). Bile acid secreted by male sea lamprey that acts as a sex pheromone. *Science* **296**, 138-141.

Marui, T., Evans, R. E., Zielinski, B. S. and Hara, T. J. (1983). Gustatory responses of the rainbow trout (*Salmo gairdneri*) palate to amino acids and derivatives. *J. Comp. Physiol.* **153**, 423-433.

Michel, W. C., Sanderson, M. J., Olson, J. K. and Lipschitz, D. L. (2003). Evidence of a novel transduction pathway mediating detection of polyamines by the zebrafish olfactory system. *J. Exp. Biol.* **206**, 1697-1706.

Nikonov, A. A. and Caprio, J. (2001). Electrophysiological evidence for a chemotopy of biologically relevant odors in the olfactory bulb of channel catfish. *J. Neurophysiol.* **86**, 1869-1876.

Polkinghorne, C. N., Olson, J. M., Gallaher, D. G. and Sorensen, P. W. (2001). Larval sea lampreys release two unique bile acids to the water at a rate sufficient to produce detectable riverine pheromone plumes. *Fish Physiol. Biochem.* **24**, 15-30.

Rolen, S. H. and Caprio, J. (2007). Processing of bile salt odor information by single olfactory bulb neurons in the channel catfish. *J. Neurophysiol.* **97**, 4058-4068.

Rolen, S. H., Sorensen, P. W., Mattson, D. and Caprio, J. (2003). Polyamines as olfactory stimuli in the goldfish, *Carassius auratus*. *J. Exp. Biol.* **206**, 1683-1696.

Sola, C. and Tosi, L. (1993). Bile salts and taurine as chemical stimuli for glass eels, Anguilla anguilla: a behavioral study. *Environ. Biol. Fishes* **37**, 197-204.

Sorensen, P. W. and Caprio, J. (1998). Chemoreception. In *The Physiology of Fishes* (ed. D. H. Evans), pp. 251-261. Boca Raton: CRC.

Sorensen, P. W., Fine, J. M., Dvornikovs, V., Jeffrey, C. S., Shao, F., Wang, J., Vrieze, L. A., Anderson, K. R. and Hoye, T. R. (2005). Mixture of new sulfated steriods functions as a migratory pheromone in the sea lamprey. *Nat. Chem. Biol.* **1**, 324-328.

Sveinson, T. and Hara, T. J. (2000). Olfactory sensitivity and specificity of Artic char, *Salvelinus alpinus*, to a putative male pheromone, prostaglandin $F_{2\alpha}$. *Physiol. Behav.* **69**, 301-307.

Vrieze, L. A. and Sorensen, P. W. (2001). Laboratory assessment of the role of a larval pheromone and natural stream odor in spawning stream localization by migratory sea lamprey (*Petromyzon marinus*). *Can. J. Fish. Aquat. Sci.* **58**, 2374-2385.

Yamashita, S., Yamada, T. and Hara, T. J. (2006). Gustatory responses to feeding- and non-feeding-stimulant chemicals, with an emphasis on amino acids, in rainbow trout. *J. Fish Biol.* **68**, 783-800.

Zeng, C. and Hidaka, I. (1990). Single fiber responses in the palatine taste nerve of the yellowtail *Seriola quinqueradiata*. *Nippon Suisan Gakkai Shi* **56**, 1611-1618.

Zhang, C., Brown, S. B. and Hara, T. J. (2001). Biochemical and physiological evidence that bile acids produced and released by lake char (*Salvelinus namaycush*) function as chemical signals. *J. Comp. Physiol., B* **171**, 161-171.