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Specificity and sensitivity of the olfactory organ of the zebrafish, *Danio rerio*

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Abstract 1. The specificity and sensitivity of the olfactory organ of adult zebrafish, *Danio rerio*, to selected amino acid, bile acid, and steroid odorants were characterized using the electro-olfactogram recording technique. The olfactory organ was responsive to 28 of the 29 odorants tested.

2. All of the 100 μM amino acid and bile acid stimulants elicited a negative-going response that was significantly greater than the response to the artificial freshwater control. The general pattern of relative stimulatory effectiveness established for the amino acid stimuli was neutral amino acids > basic amino acids > acidic amino acids > imino acids. The general pattern of relative stimulatory effectiveness of 100 μM bile acid stimuli was taurine-conjugated bile acids > glycine-conjugated bile acids ≈ non-conjugated bile acids. The responses to the most stimulatory bile acid odorants were up to 40% larger than the responses to the most stimulatory amino acid odorants.

3. The response threshold for cysteine and taurocholic acid, the most stimulatory of the amino acid and bile acid stimuli tested, was approximately 10^{-8} M. Females are significantly more sensitive to these odorants than males.

Key words Olfaction · Amino acids · Bile acids · Zebrafish · Electrophysiology

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Introduction

The vertebrate olfactory system is capable of detecting and discriminating thousands of potential odors. Electrophysiological studies indicate that individual olfactory receptor cells respond to many odorants and cannot be classified on the basis of these responses (Holley 1991). Understanding the molecular genetic and developmental processes that result in a complement of olfactory receptor neurons each with a unique response spectrum is an area of particularly active investigation.

The zebrafish, *Danio rerio*, is becoming an increasingly popular model for investigations of vertebrate development (Kimmel 1993; Strähle and Blader 1994; Rossant and Hopkins 1992). Behavioral investigations confirm that zebrafish use chemosensory information. The amino acid alanine is an attractant (Steele et al. 1990, 1991). A partially-characterized cholesterol ester (Algranati and Perlmutter 1981), released into tank water by male and female zebrafish, acts as an aggregating pheromone to both sexes (Bloom and Perlmutter 1977). An ovarian steroid glucuronide(s) initiates male courtship behaviors (Van Den Hurk and Lambert 1983) and a testicular steroid glucuronide(s) induces ovulation (Van Den Hurk et al. 1987). Zebrafish rendered anosmic fail to ovulate (females) or initiate courtship (males) indicating that at least some of these responses are olfactory-mediated. Recently, the development of the zebrafish olfactory organ (Hansen and Zeiske 1993) and organization of the olfactory bulb (Baier and Korsching 1994) were described. A family of olfactory receptor genes, similar to those previously identified in mammals (Raming et al. 1993; Buck and Axel 1991) and channel catfish (Ngai et al. 1993), was identified in zebrafish (Korsching and Baier 1992; Byrd et al. 1994). Before determining the generality of the zebrafish model for understanding fish olfaction, and to complement the advances made in the morphological

and genetic aspects of zebrafish olfactory development, the specificity and sensitivity of the olfactory organ must be physiologically established. Towards addressing this deficiency, electro-olfactogram recordings were obtained from the zebrafish olfactory organ to a variety of amino acid, bile acid and steroid odorants.

Materials and methods

Animal maintenance

Zebrafish (*Danio rerio*, formerly *Brachydanio rerio* (Meyer et al. 1993)) were purchased from a commercial supplier (Steve Lambourne Co, Inc., Sylmar, CA, USA) and housed in mixed sex populations in recirculating 40–80 l aquaria (26°C). The maximum density was 1 fish per 2 liters. The aquaria were equipped with standard undergravel/charcoal filtration systems and approximately half of the water was replaced weekly with deionized water. Normal fluorescent laboratory lighting (approximately 12:12 light:dark) was supplemented with ambient light from south-facing windows. The fish were fed flake food (Tetramin) daily. Fish were used within 6 months of arrival in the laboratory.

Prior to beginning a recording session, the fish were immobilized with an intramuscular injection of Flaxedil (60 μg/g body weight) and secured to a silastic-polymer (Sylgard) recording chamber. The olfactory epithelium was immediately provided with a continuous flow of artificial freshwater (AFW; see solutions for composition). A separate flow of ca. 3 ml/min of AFW containing the general anaesthetic MS-222 (20 mg/l in AFW) across the gills provided general anaesthesia. The olfactory epithelium was at no time exposed to the general anaesthetic. Anaesthesia was not provided prior to immobilization to minimize the loss of afferent sensory activity associated with topical application of the anaesthetic to the olfactory epithelium (Spath and Schweickert 1977). The level of anaesthesia was monitored by observation of reflexive movements of the gills and eyes. Additional anaesthetic and neuromuscular block were administered as required. After allowing at least 10 min for the general anaesthetic to act, the small flap of epithelium covering the olfactory rosette was surgically removed. Throughout this procedure, and for the duration of the experiment, the olfactory epithelium was provided with a continuous flow of AFW.

Determination of fish sex

Fish were preliminarily sexed by visual inspection before each experiment. Mature females have a plumb, pale-white abdomen. Mature males have a yellow and thin abdomen. After each experiment, while still under the influence of general anaesthetic, the weight and total length of the fish were recorded and the fish were killed by decapitation. To confirm the sex of each fish, the body cavity was opened and the presence of ovaries or testes was noted under a dissecting microscope. The presence of oocytes of 0.3–0.8 mm in diameter indicated mature female zebrafish (Selman et al. 1993, 1994). We made no further attempt to characterize the reproductive status of the fish used in this study. Under similar holding conditions, female zebrafish showed a 4–5 day reproductive cycle and males showed continuous spermatogenesis (Van Den Hurk et al. 1987). Some fish were stored in cold 4% paraformaldehyde after decapitation prior to determination of sex.

Electrophysiological methods

The electro-olfactogram (EOG) technique used to monitor the olfactory responses was virtually identical to techniques previously used in other studies of fish olfaction (e.g., Silver et al. 1976; Caprio 1978). The method records the summed receptor potentials of the olfactory receptor neurons as a characteristic negative DC voltage potential shift. The recording electrode was positioned just above and between adjacent olfactory lamellae near the midline raphe. The differential electrode was placed on the skin of the head. Both the recording and differential electrodes were fabricated from silver/silver chloride wire bridged to the fish via 3 *M* KCl/agar (1–3%) electrodes with tip diameters of ca. 10–20 μm. A silver/silver chloride ground electrode was positioned in the AFW bath. Responses to olfactory stimuli were initially amplified (1000–10000x gain) and filtered (1–2 kHz) by a low-noise differential DC amplifier. The amplified responses were displayed on an oscilloscope and also digitized (100 Hz), stored, and displayed on MS-DOS-based computer using hardware and software from Axon Instruments (Digidata 1200 A/D board and Axotape software). Prior to beginning an experiment, the recording electrode was positioned to maximize the response to 100 μ*M* Cys. Preparations were rejected if either a stable baseline could not be obtained or 100 μ*M* Cys did not elicit a response greater than 200 μV. Baseline stability problems were generally due to the introduction of bubbles into the KCl/agar bridging electrodes or inadequate silver chloride on the silver lead wires. When baseline instability was noted, fish were generally replaced because the manipulations associated with replacing electrodes and isolating the source of the baseline instability often resulted in intermittent drying of the olfactory epithelium. Fewer than 10% of the animals were rejected because of small initial responses to 100 μ*M* Cys. Occasional bleeding after injection of the neuromuscular blocker into these small fish may have contributed to some of these failures. No attempt was made to sex fish that failed to yield adequate responses.

Odorant delivery

The carrier flow of AFW bathing the olfactory epithelium was supplied from an air-pressurized (3 psi) polyethylene bottle and regulated to 3 ml/min with a teflon and glass flowmeter (Cole Parmer). The tubing and connectors of the olfactometer were made of teflon or polyethylene. A piece of 18 gauge stainless steel tubing directed the output flow over the olfactory epithelium. Olfactory stimuli were introduced into carrier flow of AFW bathing the olfactory epithelium through a rotary loop injector (Rheodyne Inc). The volume of odorant injected was ca. 50 μl. Photometric calibration of the olfactometer flow was accomplished by placing a matched LED and photodiode across the flow and injecting dye (filtered non-dairy creamer, 5 g/100 ml de-ionized water). This calibration indicated that a bolus of odor arrived at the preparation ca. 6 s after injection, reached a peak concentration of 84% ($\pm$ 0.07%, SD, $n = 3$) of the loaded concentration by 8 s, and decayed to the baseline concentration after *ca.* 12 s. Direct calibration of the concentration of odorant contacting the olfactory organ adjacent to the recording electrode was not attempted. Reported concentrations have not been corrected for dilution.

Experimental protocols

Determination of the specificity and sensitivity of the zebrafish olfactory organ began after a stable response to 100 μ*M* Cys was obtained. For all experiments, each odorant (or odorant concentration) was sequentially tested at least twice. At least 2 min were allowed between stimulus injections. After every 6–8 stimulants, 100 μ*M* Cys and the AFW control were retested to confirm the stability of the preparation. The order of odorant presentation was changed for each fish tested with the exception that concentration-response functions were always determined by testing odors from lowest to highest concentration. The amino acid specificity, steroid/bile acid specificities and concentration response functions of the

olfactory organ were each determined in different experiments using separate groups of fish.

Solutions

All solutions were prepared in deionized water with a resistivity > 18MΩ-cm. The composition of the AFW was (in m*M*) NaCl 3; KCl 0.2; $CaCl_2$ 0.2; HEPES 1 - pH 7.2). A 10 m*M* stock solution of each amino acid was prepared in AFW biweekly and stored at 4°C until use. Stock solutions of water-soluble bile acids and steroids were prepared at either 1 m*M* or 10 m*M* in AFW biweekly and stored at 4°C until use. Several of the bile acids and steroids are relatively insoluble in water and were prepared at 10 m*M* in absolute ethanol and stored at − 20°C until use. For these bile acids, ethanol, appropriately diluted in AFW, served as the control (EtOH). All odorants were purchased from Sigma Chemical Company.

Data analysis

The magnitude of the olfactory response to each odorant for each fish was calculated as the average of the peak responses, measured in millivolts (mV), of all individual presentations of an odorant. A mean response of all fish to an odorant was calculated from the average responses of the individual fish. The first response to glutamate (Glu) had a second negative component that often obscured the peak of the first component. When the peak of the first response component could not be resolved, the response to Glu was calculated from the second and third Glu applications, where the second component was either much smaller or absent. A *z*-test (for large-sample approximations) was used to compare the responses of male and female fish to the first cysteine application (Zar 1984). Specificity and sensitivity data were analyzed using 2-way ANOVA following a square-root transformation to meet the assumptions of the test (Zar 1984). In cases where each fish was subjected to a number of treatments, such as different concentrations of an odorant, comparisons between responses were analyzed using ANOVA for repeated measures. A posteriori Tukey tests were used to compare the responses to odorants with the responses to the AFW or EtOH when the ANOVA identified significant differences between odorants or concentrations. A posteriori *t*-tests were used to compare the responses of males and females to specific odorants or concentrations when the ANOVA identified a significant interaction between these variables and the sex of the fish. The response threshold was established as the lowest concentration of odorant eliciting a response significantly greater than the response to the AFW or EtOH control.

Results

The olfactory responses of 52 female and 37 male zebrafish are considered in the current investigation. The average weight and body length of the females used in this study were slightly, but significantly, larger than the male measurements (*z*-test, $P < 0.05$ for both variables). The average weights of female and male zebrafish were 0.70 ± 0.02 (SEM) g and 0.59 ± 0.02 g, respectively. The average length of female and male zebrafish measured from the snout to the caudal peduncle were 3.4 ± 0.05 cm and 3.3 ± 0.05 cm, respectively.

All odorants elicited a slow, negative DC potential change that varied in magnitude as a function of the odorant and concentration tested (Fig. 1). The only stimulus that routinely elicited a different pattern of response was Glu. The first application of 100 μ*M* Glu elicited a response with an extra, slower negative component from 34 of the 35 fish tested (Fig. 2). The time to first peak of the response occurred at the same time as the peak response elicited by other odorants. The slower, second component reached its maximum magnitude 3.7 ± 1.7 ($n = 35$, SD) s after the first peak. The second component of the Glu response was labile. Repeated stimulation with 100 μ*M* Glu substantially reduced or eliminated the second component. After a prolonged recovery period (> 10 min) the second response component returned (data not shown).

Each fish was initially tested with 100 μ*M* Cys to optimize this response during the placement of the electrodes. The average Cys response of female zebrafish of 1.51 ± 0.07 (SEM, $n = 52$) mV was significantly greater than the average response of male zebrafish of 0.98 ± 0.06 mV ($n = 37$; z-test, $P < 0.05$). There was no significant correlation between magnitude of the Cys response and either fish weight or length for either sex. EOG data are often normalized to a standard odorant to minimize variability in response magnitude that may be due to systematic increases in response magnitude during long experiments and/or a result of inter-fish variability. Sex-based differences in olfactory specificity or sensitivity would be eliminated by such a standardization procedure if such differences were of equal magnitude for all odorants so results were not standardized.

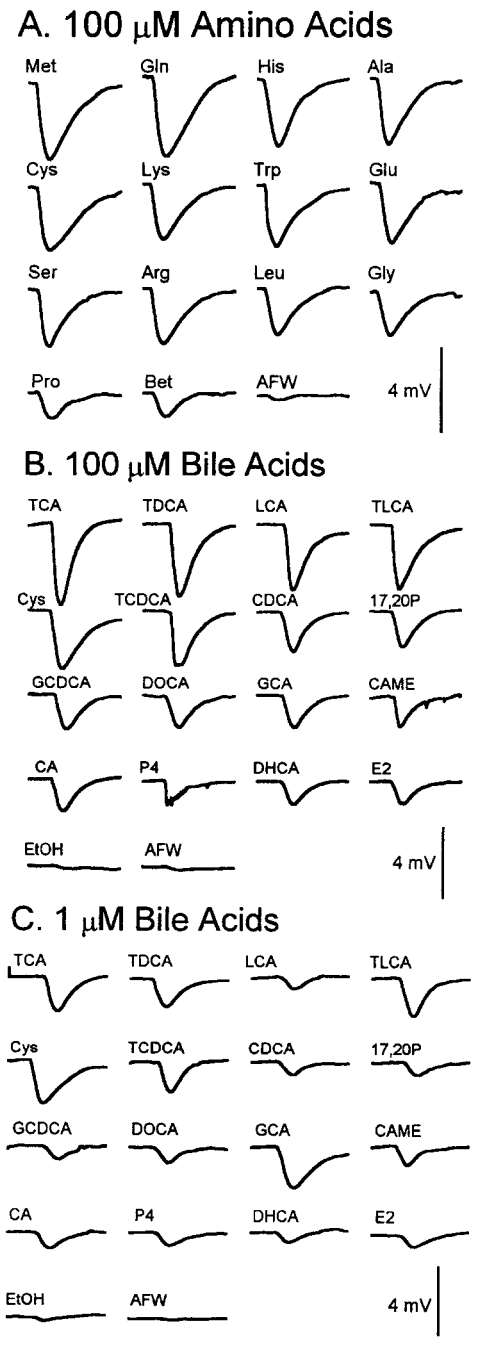
Amino acid specificity

The effectiveness of 100 μ*M* concentrations of 14 amino acid stimuli was determined for 8 male and 13 female zebrafish (Figs. 1A, 3A). A 2-way ANOVA for repeated measures ($P < 0.05$) identified a significant odor main effect indicating that differences in amino acid effectiveness exist. The ANOVA indicated a non-significant sex main effect and a non-significant interaction between sex and odor, suggesting that sex was not a critical factor. A posteriori Tukey tests, to determine the cause of the significant odor effect, indicated that the responses to all amino acid stimuli were significantly greater than the response to the AFW control. At 100 μ*M*, the relative stimulatory effectiveness was neutral amino acids > basic amino acids > Glu (an acidic amino acid) > proline (an imino acid).

Bile acid specificity

The responses of zebrafish to 12 bile acid and 3 steroid odorants were obtained at concentrations of 100 μ*M*,

1 μ*M*, and 0.01 μ*M* (Figs. 1B,C,4). At each of these concentrations the 2-way ANOVA for repeated measures identified an odor main effect but failed to identify a sex main effect or an interaction between odor and sex. At 100 μ*M* all of the bile acids were more stimulatory than the appropriate control (AFW for bile acid stocks prepared in AFW, ethanol (EtOH) appropriately-diluted with AFW for bile acid stocks prepared in EtOH). The relative stimulatory effectiveness of bile acid stimuli was taurine-conjugated bile acids > glycine-conjugated bile acids ≈ non-conjugated bile acids. At a test concentration of 1 μ*M*, 9 of the 12 bile acids elicited a response that was significantly greater than the response to the appropriate control (Tukey test, $P < 0.05$). The 4 taurine-conjugated bile acids and the 2 glycine-conjugated bile acids were significantly more effective than the AFW control. The most effective bile acid tested at 1 μ*M* concentration was GCA. At 0.01 μ*M*, the average responses of 9 of 12 bile acid stimuli were significantly greater than the appropriate control (AFW or EtOH). The responses to 100 μ*M* and 0.01 μ*M* 4-Pregnene-17α,20β-diol-3-one (17,20P) and 0.01 μ*M* progesterone (P4) were significantly greater than the response to the EtOH control. None of the responses to the tested concentrations of β-estradiol (E2) were significantly different from the responses to the appropriate EtOH control.



Concentration response characteristics

The sensitivity of zebrafish was investigated by determining the concentration-response characteristics of 3 amino acids, Cys, arginine (Arg), and Glu, and 2 bile acids, TCA, and chenodeoxycholic acid (CDCA) (Fig. 5). Each of these odorants was selected on the basis of preliminary cross-adaptation experiments indicating that each interacts with a distinct receptor site (unpublished data). A significant concentration main effect was identified for each odorant (2-way ANOVA for repeated measures, $P < 0.05$). Significant interactions between sex and concentration were identified for both TCA and Cys (2-way ANOVA for repeated measures, $P < 0.05$). The sex-based difference in sensitivity was reflected in a lower female detection threshold for these two odorants and significantly larger female responses to several of the suprathreshold concentrations of these odorants. The detection thresholds for female and male zebrafish, established as the lowest

Fig. 1 The zebrafish olfactory organ responds to a broad range of aqueous odorants. EOG responses of two adult female zebrafish to (**A**) 100 μ*M* amino acid, (**B**) 100 μ*M* bile acid, and (**C**) 1 μ*M* bile acid stimuli were recorded. Each trace is the same 16 s portion of the 40 s digitally reconstructed record obtained for each odorant. Responses obtained in B and C are from the same fish. *Abbreviations* Amino acid odorants: *Ala* L-alanine; *Arg* L-arginine; *Bet* betaine; *Cys* L-cysteine; *Glu* L-glutamate; *Gln* L-glutamine; *Gly* glycine; *His* L-histidine; *Leu* L-leucine; *Lys* L-lysine; *Met* L-methionine; *Pro* L-proline; *Ser* L-serine; *Trp* L-tryptophan. Bile acid and steroid odorants prepared from ethanol stock solutions: *CA* Cholic acid; *CAME* Cholic acid methyl ester; *CDCA* Chenodeoxycholic acid; *DHCA* Dehydrocholic acid; *DOCA* Deoxycholic acid; *E2* β-estradiol; *LCA* Lithocholic acid; *17,20P* 4-Pregnene-17α,20β-diol-3-one; *P4* Progesterone. Bile acids prepared from aqueous stock solutions: *GCA* Glycocholic acid; *GCDCA* - Glycochenodeoxycholic acid; *TCA* Taurocholic acid; *TCDCA* Taurochenodeoxycholic acid; *TDCA* Taurodeoxycholic acid; *TLCA* Taurolithocholic acid 3-Sulfate. Control solutions: *AFW* artificial freshwater; *EtOH* AFW-diluted ethanol control

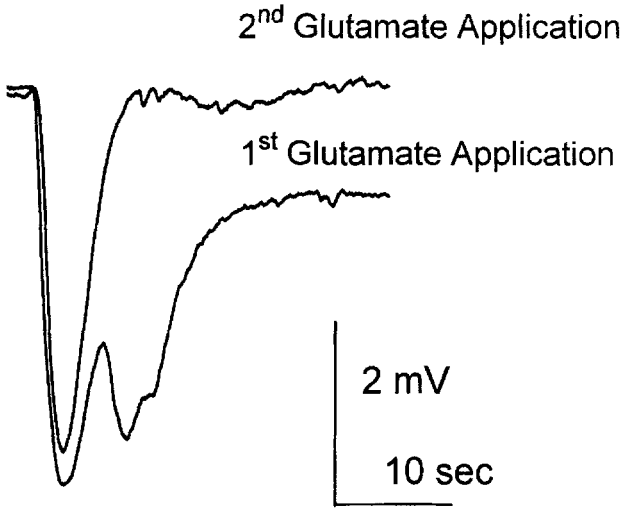


Fig. 2 Representative example of the bimodal response elicited by the first application of 100 μ*M* Glu and the subsequent reduction or elimination of the second component upon repeated Glu stimulation. The two traces are identical portions of the 40 s digitally reconstructed records obtained for the first and second sequential presentations of 100 μ*M* Glu tested at a 2 min interval

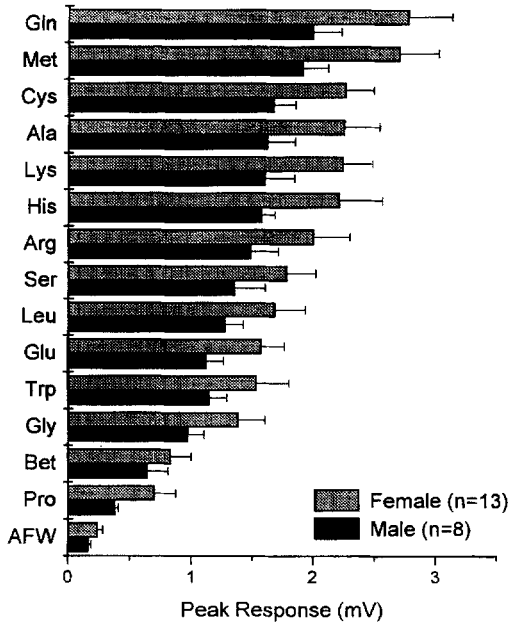


Fig. 3 The olfactory organ of adult zebrafish is sensitive to amino acid stimuli. The average peak responses (± SEM) of the 13 female and 8 male zebrafish olfactory organs to 100 μ*M* amino acid stimuli are plotted. The average responses of both sexes to all of the amino acid odorants were significantly greater than the average response to AFW (ANOVA - $P < 0.05$ followed by Tukey tests). *Abbreviations* as in Fig. 1

TCA or Cys concentration eliciting a response significantly greater than the response to the AFW control, were 0.01 μ*M* and 0.1 μ*M*, respectively. For Arg, Glu and CDCA, the ANOVA identified a significant concentration main effect but the sex main effect and interaction between sex and concentration were not significant. The estimated detection thresholds established for these odorants were 0.1 μ*M* for Arg, 1 μ*M* for Glu, and 1 μ*M* for CDCA.

Discussion

This investigation establishes that the olfactory organ of the zebrafish responds to a broad range of odorants. The specificity of the zebrafish olfactory organ to amino acid stimuli is generally similar to that of other fishes that have been examined. Like several related cyprinid fishes (Zippel et al. 1993; Johnsen and Adams 1986; Goh et al. 1979; Goh and Tamura 1978), the zebrafish olfactory organ is nearly equally sensitive to basic amino acids and neutral amino acids. In contrast, the olfactory organs of several catfish (Caprio 1978, 1980) and salmonid (Hara 1977a, b; Hara 1976; Hara et al. 1973) species are relatively less sensitive to basic amino acids compared to neutral amino acid stimuli. Our finding that the taurine-conjugated bile acids are generally more effective odorants than either the glycine-conjugated, or non-conjugated forms, indicates that the relative effectiveness of bile acids for zebrafish is similar to the specificity reported in other fish species (Doving et al. 1980; Thommesen 1983; Hara et al. 1984; Zhang and Hara 1994). The steroid 17,20P, which acts as a reproductive pheromone and odorant in goldfish (Sorensen et al. 1987), crucian carp (Bjerselius and Olsen 1991) and common carp (Irvine and Sorensen 1993), elicited a slightly, but significantly, larger response than the EtOH control from the zebrafish olfactory organ. Given that pheromones generally confer species specificity, it is not surprising that neither catostomids (Cardwell et al. 1992) nor zebrafish (current study) are particularly sensitive to the specific reproductive pheromones used by goldfish. Perhaps zebrafish-specific analogs of the steroid pheromones would elicit larger responses. Alternatively, sensitivity to a putative pheromone may require priming with other substances. For example, precocious male Atlantic salmon parr require pre-exposure to the urine of ovulated females before 17α,20β-dihydroxy-4-pregnen-3-one 20-sulphate becomes a potent odorant (Moore and Scott 1992). Mature goldfish are more sensitive to reproductive pheromones than juveniles (Sorensen et al. 1987). Although the precise point in the 4–5 day reproductive cycle was not determined (Van Den Hurk et al. 1987), the presence of oocytes of 0.3–0.8 mm in diameter indicates that our EOG recordings were obtained from mature female zebrafish (Selman et al. 1994, 1993). Many of the male zebrafish tested in the present study were also likely to be mature since males of similar size tested in another study (Van Den Hurk et al. 1987) showed continuous spermatogenesis. Further, zebrafish of similar size were bred in our laboratory.

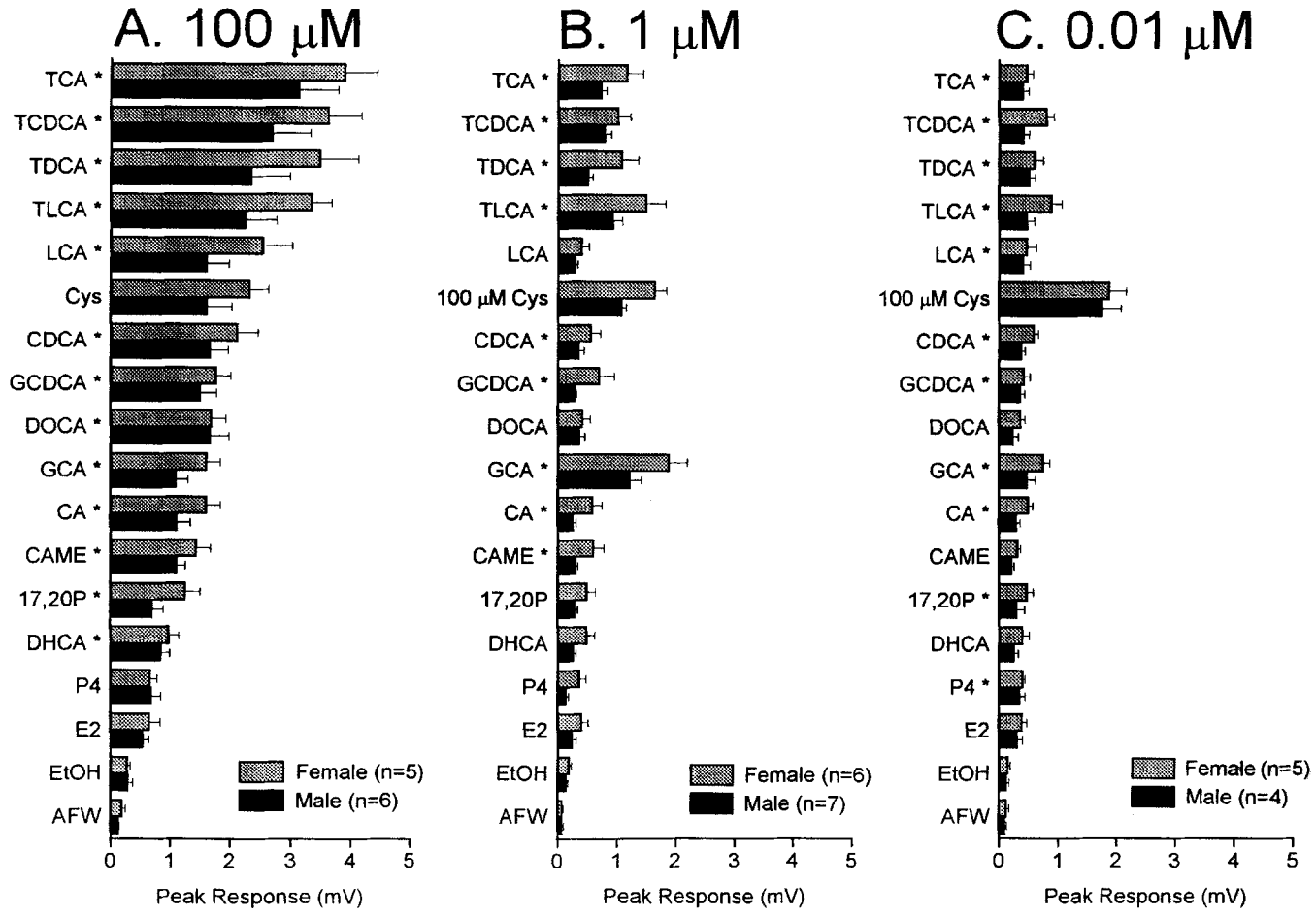


Fig. 4A–C The olfactory organ of adult zebrafish is sensitive to bile acid stimuli. Average peak responses (± SEM) of female and male zebrafish to bile acid and steroid stimuli tested at concentrations of 100 μ*M* (**A**), 1μ*M* (**B**) and 0.01 μ*M* (**C**) are plotted. The average response to 100 μ*M* cysteine is also shown. Since Cys has already been shown to be more effective than the AFW control (Fig. 3) and was not tested at the same concentration as the bile acids it was excluded from the statistical comparisons of the bile acids. An *asterisk* to the right of the odorant abbreviation designates an average response significantly greater than the appropriate control (Since the sex main effect was not significant, the average of pooled male and female responses to odorants are compared with control responses). The numbers of male and female fish tested at each concentration are indicated in *parentheses* next to the legend. *Abbreviations* as in Fig. 1

The zebrafish olfactory organ responded to amino acids at threshold concentrations similar to those reported for other fishes, ranging from 10^{-7} *M* to 10^{-9} *M* (Hara 1992). The response thresholds of the zebrafish olfactory organ to bile acids were also similar to those reported for goldfish (Sorensen et al. 1987) and rainbow trout (Hara et al. 1984), ranging from 10^{-6} *M* to 10^{-9} *M*. Several bile acids, naturally-synthesized and released by juvenile sea lamprey, were detected at sub-picomolar concentrations by adult sea lamprey (Li and Sorensen 1994). Since the commercially-supplied bile acids used in the current study were isolated from mammalian sources, it remains to be determined if bile acids synthesized and released from zebrafish might be more stimulatory odorants.

Differences in the responses of female and male zebrafish to TCA and Cys provide compelling evidence for sex-based differences in olfactory sensitivity to these non-pheromonal odorants. The average female response to the application of 100 μ*M* Cys that began each experiment was significantly larger than the corresponding male response. The lack of correlation between fish size and the response magnitude to 100 μ*M* Cys indicated that the sex-based difference in olfactory sensitivity was not due to the slightly larger size of females. The suprathreshold responses of female zebrafish to all tested concentrations of TCA and all but one concentration of Cys (10 μ*M*) were significantly greater than the corresponding responses obtained from male zebrafish. The response thresholds of female zebrafish to Cys and TCA were ca. 0.01 μ*M*. The corresponding response thresholds for male zebrafish were ca. 0.1 μ*M*. A combined histological and physiological investigation is required to determine if the olfactory sensitivity of zebrafish, particularly of females, varies as a function of time over the relatively short (4–5 day) reproductive cycle. Differences in the endocrine states

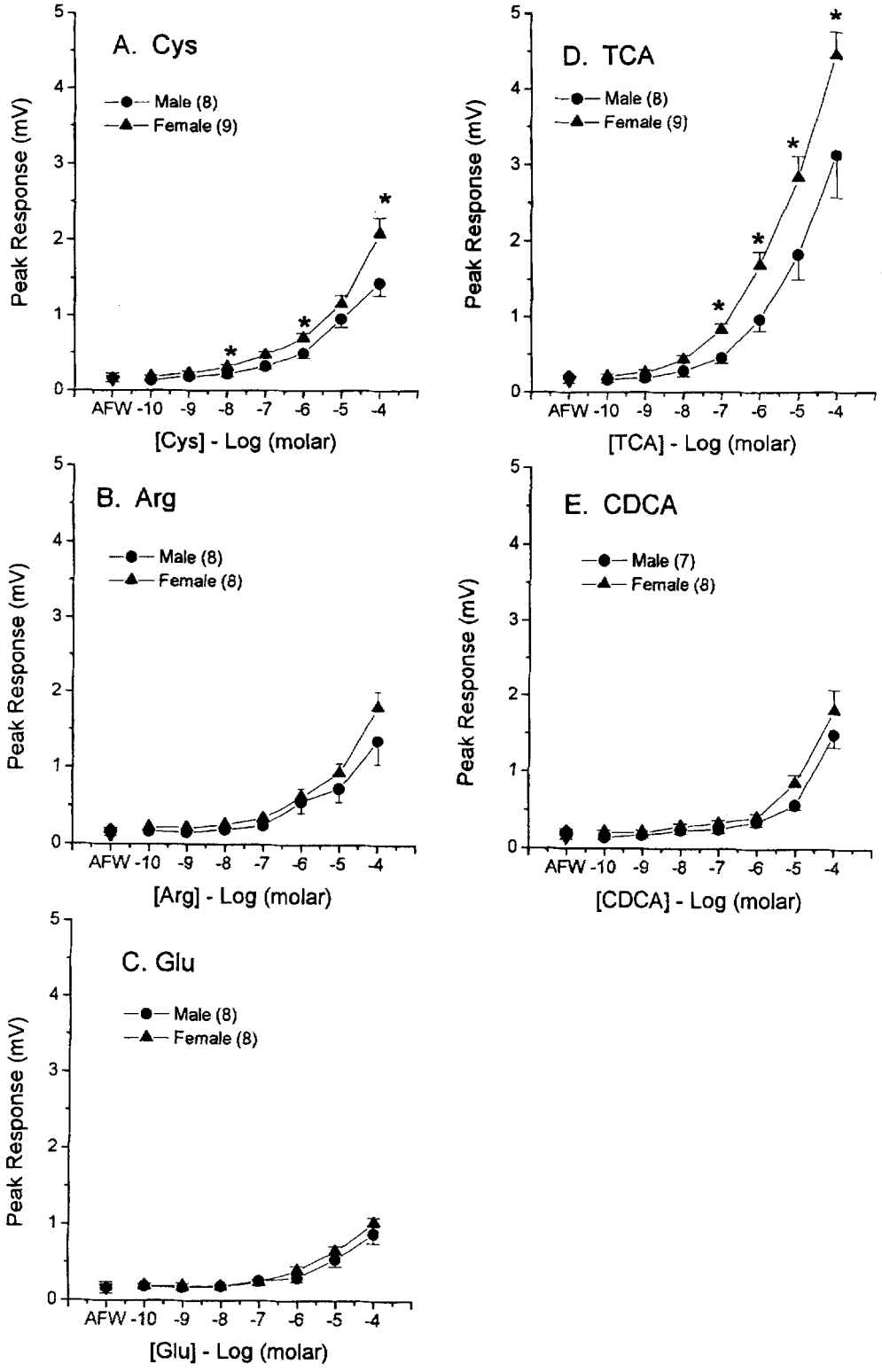


Fig. 5A–E Semi-log plots of the concentration response characteristics of 3 amino acid and 2 bile acid stimulants. Plotted are the responses of female and male zebrafish to (**A**) Cys, (**B**) Arg, (**C**) Glu, (**D**) TCA, and (**E**) CDCA. An *asterisk* designates a significant difference in the magnitude of the female and male responses at the indicated concentration. *Numbers in parentheses* indicate the number of fish tested

of male, female, and juvenile common carp have been proposed as possible sources of the observed differences in the olfactory sensitivity to serine and several sex pheromones (Irvine and Sorensen 1993).

Reports of other sex-based differences in olfactory sensitivity and/or specificity are limited. Male goldfish are more sensitive to the sex pheromone prostaglandin $F_{2\alpha}$ (Sorensen et al. 1988; Sorensen and Goetz 1993),

but equally as sensitive as females to the putative pheromone 17α,20β-dihydroxy-4-pregnen-3-one (17, 20P) and several amino acids (Sorensen et al. 1987). Female fiddler crabs feed longer and respond to lower concentrations of feeding stimulants than males (Weissburg et al. 1993). These behavioral differences may be due to the lower threshold and higher firing frequency of the female chemosensory neurons to feeding stimulants (Weissburg and Derby 1994) and/or to a greater number of chemosensory neurons on the feeding claws of females (Weissburg et al. 1993). Female humans perform better at odor identification than males in all corresponding age classes (Doty 1986).

Non-neuronal response components have been observed in the EOG recordings of several species (e.g., Erickson and Caprio 1984; Getchell 1974; Okano and Takagi 1974). In the channel catfish, the non-neuronal response is reported to be an extra, slower, negative component. In this species, several bile acid and amino acid stimuli were reported to elicit both neural and non-neuronal response components (Erickson and Caprio 1984). In the zebrafish, an extra, negative component was observed in response to 100 μ*M* Glu but not to bile acids or to other amino acid odorants. The slower kinetics and labile nature of the second component of the Glu response are consistent with a non-neuronal origin. The cellular origin of the non-neuronal component remains to be determined.

The zebrafish is already widely accepted as a model for investigations of vertebrate development (Kimmel 1993; Strähle and Blader 1994; Rossant and Hopkins 1992). Since the response properties of the olfactory organ of zebrafish and most other fish examined are similar, the zebrafish appears to be an appropriate choice as a general model for investigations of olfactory development in fish. The present physiological characterization represents an initial step in providing a functional context for recent molecular and developmental investigations of zebrafish olfaction, such as the identification of olfactory receptor genes (Korsching and Baier 1992) and their expression during development (Byrd et al. 1994).

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