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Polyamines as olfactory stimuli in the goldfish *Carassius auratus*

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Summary

Electrophysiological responses of goldfish olfactory receptor neurons (ORNs) and goldfish behavioral responses to polyamines were investigated *in vivo*. Electro-olfactogram (EOG) recordings indicated that polyamines (putrescine, cadaverine and spermine) are potent olfactory stimuli for goldfish with estimated electrophysiological thresholds of 10^{-8}–10^{-7} mol l^{-1}, similar to that for L-arginine, the most stimulatory amino acid. Although thresholds were similar, the magnitude of the EOG responses to intermediate (10^{-5}–10^{-4} mol l^{-1}) and high (10^{-3} mol l^{-1}) concentrations of polyamines dwarfed the responses to amino acids and related single amine containing compounds (amylamine and butylamine). The EOG responses to 0.1 mmol l^{-1} putrescine, cadaverine and spermine were, respectively, 4.2×, 4.3× and 10.3× the response of the standard, 0.1 mmol l^{-1} L-arginine. Electrophysiological cross-adaptation experiments indicated that polyamine receptor sites are independent from those to L-amino acids (alanine, arginine, glutamate, lysine, methionine and ornithine), bile salts (sodium taurocholate and taurolithocholate), the single amine containing compounds (amylamine and butylamine) and ATP. Further, the cross-adaptation experiments revealed the existence of independent receptor sites for the different polyamines tested. Pharmacological experiments suggested that polyamine odorant transduction does not primarily involve the cyclic AMP and IP_3 second messenger pathways. Behavioral assays indicated that polyamines are attractants that elicit feeding behavior similar to that elicited by L-amino acids.

Key words: electro-olfactogram, olfaction, receptor site, second messenger, goldfish, *Carassius auratus*.

Introduction

Odorant detection and discrimination begin at the level of the olfactory receptor neuron (ORN). ORNs in vertebrates express in their apical cilia and microvilli, respectively, molecular odorant receptors encoded by a family of approximately 1000 genes in mammals (Buck and Axel, 1991), but only approximately 100 genes in teleosts (Ngai et al., 1993), which are all members of the seven transmembrane domain G protein-coupled receptor superfamily (Cao et al., 1998; Speca et al., 1999; Mombaerts, 1999).

Olfactory stimuli in tetrapods are volatile compounds, whereas those for fish are water-soluble. Further, in contrast to mammals (Raming et al., 1993; Zhao et al., 1998; Krautwurst et al., 1998; Malnic et al., 1999; Wetzel et al., 1999; Araneda et al., 2000), the ligand specificity for molecular olfactory receptors in any teleost is largely unknown; the sole exception is the goldfish L-arginine/L-lysine amino acid receptor (Speca et al., 1999). Activation of odorant receptors through ligand binding in vertebrates facilitates second messenger cascades, such as the cyclic AMP (cAMP) and IP_3 signaling pathways (Bruch, 1996; Schild and Restrepo, 1998). In mammals, however, the prevailing evidence is for the cAMP pathway (Belluscio et al., 1998; Brunet et al., 1996), whereas in fish it is the IP_3 pathway (Speca et al., 1999; Bruch, 1996). In addition, in both mammals (Xu et al., 2000) and fish (Hara and Zhang, 1996; Nikonov and Caprio, 2001; Friedrich and Korsching, 1997, 1998), ORNs expressing receptors for different classes of odorants project their axons to the olfactory bulb, forming a relatively precise odotopic map. Although numerous types of volatile chemicals are known to stimulate ORNs of tetrapods, the identification of biologically relevant classes of water-soluble odorants for fish is limited.

Amino acids, bile salts, nucleotides, gonadal steroids and prostaglandins have been previously identified as behaviorally relevant olfactory cues for teleosts, and mediate behaviors ranging from feeding and predator detection to social interactions and reproductive synchrony (Sorensen and Caprio, 1998). Information concerning other classes of chemicals that might also be olfactory stimuli of biological relevance is lacking; however, an electrophysiological survey of additional classes of water-soluble, naturally occurring chemicals in goldfish indicated that polyamines caused large olfactory

generator potentials, which are reflected in the electro-olfactogram (EOG) recordings. The present study investigates the electrophysiological (EOG and integrated neural) responses of goldfish ORNs to polyamines and whether these compounds result in changes in animal behavior.

Polyamines (putrescine, cadaverine and spermine) are naturally occurring aliphatic polycations that are widely distributed in biological materials. Intracellular putrescine and spermine concentrations have been reported in the mmol l^{-1} range for a variety of organisms (*E. coli*, rat and human) (Tabor and Tabor, 1976; Ortiz et al., 1983), although the concentration of a specific polyamine can vary with cell type, growth cycle phase and overall health of the cell. Putrescine, a precursor in spermine biosynthesis, is produced by the ornithine decarboxylase (identified in prokaryotes, fungi and mammals) and arginine decarboxylase-agmatineureohydrolase (in prokaryotes) pathways. Putrescine and spermine play key roles in an array of fundamental cellular processes, including cell growth, cell division (Tabor and Tabor, 1984) and ion channel modulation (see Discussion). In addition to their occurrence in living tissues, a previous study indicated that concentrations of putrescine, cadaverine and spermine were correlated with the degree of decomposition of certain aquatic animals (Mietz and Karmas, 1978). Since polyamine concentrations vary with degradation, and polyamines are distributed ubiquitously, teleosts are likely to encounter them in an aquatic environment. A previous investigation tested putrescine as a possible olfactory stimulus in zebrafish, but the results were negative (Fuss and Korsching, 2001).

The present study, which investigates ORN responses to polyamines, indicates that: (1) polyamines are potent olfactory stimuli to goldfish, (2) polyamine olfactory receptor sites are relatively independent of receptor sites for other known classes of odorants, (3) relatively independent receptor sites exist for different polyamines, (4) polyamine odorant information is likely to be transduced by a signaling pathway other than the classical cAMP or IP_3 cascades, and (5) polyamines are effective stimuli that promote feeding responses, similar to the behavior exhibited in response to L-amino acids. Preliminary results were previously reported in abstract form (Rolen et al., 2001, 2002).

Materials and methods

Electrophysiological experiments

Experimental animals

Goldfish, 7.5–12.5cm sexually immature shubunkins *Carassius auratus* L., were obtained from a local pet store and from the Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota (courtesy of Dr Peter Sorensen). Fish were held in the Louisiana State University Animal Care Facility in 190 l aquaria filled with charcoal-filtered tap water (CFTW) and maintained on a 12 h:12 h light:dark regime until used for experimental testing. The fish were fed a daily diet of floating commercial fish chow.

Animal preparation

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

Each goldfish was immobilized with an initial intramuscular injection of Flaxedil (gallamine triethiodide, 0.015 mg/25 g body mass). Subsequent injections of Flaxedil were provided as needed during experimentation *via* a hypodermic needle embedded in the flank musculature. After immobilization, the goldfish was wrapped in a wet Kim Wipe® and secured with lateral body clamps in a custom-made Plexiglas® container; the head was stabilized with a metal mouthpiece. The gills were irrigated *via* a constant flow of CFTW containing the general anesthetic, MS-222 (ethyl-m-aminobenzoate methane sulfonic acid, initial concentration, 0.0005%; Sigma Chemical, St Louis, MO, USA). Minor surgery was performed to remove the skin and connective tissue superficial to the olfactory rosette, facilitating electrode placement.

Stimulus solutions and delivery

The odorants included the following L-amino acids: acidic (glutamate, $–OOC\text{-}(CH_2)_2\text{-}CH(NH_3^+)\text{-}COO^–$), basic (arginine, $H_2N\text{-}C(NH_2^+)\text{-}NH\text{-}(CH_2)_3\text{-}CH(NH_3^+)\text{-}COO^–$; lysine, $^+H_3N\text{-}(CH_2)_4\text{-}CH(NH_3^+)\text{-}COO^–$ and ornithine, $^+H_3N\text{-}(CH_2)_3\text{-}CH(NH_3^+)\text{-}COO^–$), short side-chain neutral (alanine, $H_3C\text{-}CH(NH_3^+)\text{-}COO^–$), and long side-chain neutral (methionine, $H_3C\text{-}S\text{-}(CH_2)_2\text{-}CH(NH_3^+)\text{-}COO^–$), together with amines (putrescine, $H_3N^+\text{-}(CH_2)_4\text{-}NH_3^+$; cadaverine, $H_3N^+\text{-}(CH_2)_5\text{-}NH_3^+$; spermine, $H_3N^+\text{-}(CH_2)_3\text{-}NH_2^+\text{-}(CH_2)_4\text{-}NH_2^+\text{-}(CH_2)_3\text{-}NH_3^+$; butylamine, $H_3N^+\text{-}(CH_2)_3\text{-}CH_3$ and amylamine, $H_3N^+\text{-}(CH_2)_4\text{-}CH_3$), bile salts [sodium salts of taurocholic acid (TCA; $C_{26}H_{44}NO_7SNa$) and taurolithocholic acid (TLCA; $C_{26}H_{44}NO_5SNa$)], ATP ($C_{10}H_{14}N_5O_{13}P_3Na_2$) and glutaric acid ($–OOC\text{-}(CH_2)_3\text{-}COO^–$). All chemical stimuli were purchased from Sigma (St Louis, MO, USA) and were of the highest purity available. Stock solutions of amino acids, glutaric acid and amines were prepared weekly using CFTW; bile salts and ATP were prepared using Milli Q water (resistivity, 18.2 MΩ cm^{-1}). All stock solutions were pH adjusted to match control CFTW (pH 8.7) bathing the olfactory mucosa and refrigerated when not in use. Stock solutions of ATP were frozen (–20°C) in 1 ml portions for up to one month. Stock solutions were diluted daily to experimental concentrations (10^{-3}–10^{-8} mol l^{-1}) with CFTW. Analysis of the CFTW by the Dionex AAA-Direct Amino Acid Analysis System (Sunnyvale, CA, USA) indicated that no free amino acids were present (sensitivity was in the mid femtomole to low picomole range).

Stimulus delivery was *via* a 'gravity-feed' system previously described (Sveinson and Hara, 2000). Briefly, stimulus solutions and the CFTW used to bathe the olfactory epithelium were delivered through separate Teflon® tubes (diameter 0.8 mm) to the olfactory mucosa at a flow rate of 5–7 ml min^{-1}. 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 3 s applications. CFTW continuously perfused the olfactory mucosa to (1) prevent the mucosa from desiccating, (2) facilitate stimulus delivery, (3) avoid the introduction of mechanical artifacts associated with stimulus presentation and (4) rinse the olfactory organ clear of any residual stimuli for a minimum of 2 min between stimulus applications.

Pharmacological agents

Forskolin (an adenylate cyclase activator; Sigma Chemical, St Louis, MO, USA) and 1,9-dideoxyforskolin (inactive analog of forskolin; Calbiochem, La Jolla, CA, USA) were dissolved in dimethyl sulfoxide (DMSO) and added to CFTW to provide 10^{-4} mol l^{-1} stock solutions. Forskolin and 1,9-dideoxyforskolin were refrigerated when not in use for up to 1 week during experimental testing. U-73122, a potent inhibitor of agonist-induced phospholipase C (PLC) activation (Yule and Williams, 1992) and U-73343, a weak inhibitor of agonist-induced PLC activation (purchased from Biomol Research Laboratories, Inc., Plymouth Meeting, PA, USA) were prepared in the same manner as forskolin and frozen at –20°C when not in use. DMSO controls were adjusted in concentration to match those used to dissolve the pharmacological agents.

Electrophysiological recording techniques

The underwater EOG, a slow DC potential change in the water above the olfactory mucosa, is suggested to be the summed generator potentials of the responding ORNs in response to odorant molecules (Ottoson, 1971; Caprio, 1995). EOG recordings were obtained *in vivo* with calomel electrodes *via* Ringer's-agar-filled capillary pipettes. The pipette of the active electrode was positioned near the midline raphe of the olfactory rosette at a location that maximized the EOG response to 0.1 mmol l^{-1} L-arginine; the pipette of the reference electrode was placed against the skin adjacent to the olfactory cavity. The fish was grounded *via* a hypodermic needle inserted into the flank musculature. The EOG was amplified (Grass P-18; Astro-Med Inc., West Warwick, RI, USA), displayed on an oscilloscope and DC chart recorder. During the experiments, the standard (0.1 mmol l^{-1} L-arginine) was applied intermittently; if the responses to the bracketed standard differed by >25%, those data were excluded from subsequent analysis.

In vivo recordings of multiunit ORN activity were made using metal-filled glass capillary electrodes plated with platinum (Pt) (ball diameter, approx. 18–25 μm; cross-sectional area approx. 250–500 μm^2; impedance, 10–40 KΩ) placed against the sensory face of an olfactory lamella (Gesteland et al., 1959; Caprio, 1995). The electrode was r.c.-coupled (220 pF capacitor, 20 MΩ resistor) to a high-impedance probe at one input with the other input grounded *via* a hypodermic needle embedded in the flank musculature of the fish. The multi-unit neural activity was amplified (Grass P511; bandpass 30–300 Hz), observed on an oscilloscope, integrated (0.5 s) and displayed using a pen recorder.

Cross-adaptation paradigm

Electrophysiological cross-adaptation experiments to determine the relative independence of receptors for the odorant stimuli consisted of three stages: pre-adaptation, adaptation and post-adaptation.

During pre-adaptation, CFTW continuously bathed the olfactory mucosa for a minimum of 10 min prior to stimulus application. Initially, the concentrations of the test stimuli were adjusted to provide approximately equal EOG-response magnitude. Some cross-adaptation experiments involved mixtures of odorants, in which case the concentration of each component of a stimulus mixture was also adjusted to provide an approximately equal EOG-response magnitude when tested individually. The adjusted concentrations of the test stimuli ensured that potent and weak stimuli were approximately equipotent. CFTW served as the control during pre-adaptation.

During adaptation, the adapting solution at the previously adjusted concentration continuously bathed the olfactory mucosa for a minimum of 10 min prior to stimulus application. All stimuli tested during the adaptation paradigm were dissolved in the adapting solution. Controls were portions of the adapting solution and CFTW, respectively. Adaptation to an odorant suppressed the EOG responses to varying degrees to some test stimuli while not affecting the responses to others. Responses to test stimuli that were suppressed to the control level (complete adaptation) were considered to share the same receptor site(s) and/or the same transduction process as the adapting stimulus. Responses to test stimuli significantly greater than the control level were considered to have at least partially independent receptor site(s) and/or transduction processes from the adapting stimulus.

During post-adaptation, CFTW continuously bathed the olfactory mucosa for 10 min prior to stimulus application. Stimuli and controls were identical to those described during pre-adaptation.

Statistical analysis

Statistically significant differences between groups were determined by a one-way analysis of variance (ANOVA) with StatMost Version 3.5 (2001; Dataxiom Software Inc., Los Angeles, CA, USA). Means were further analyzed using the Tukey *post hoc* test. $P<0.05$ was accepted as a statistically significant difference. A student's *t*-test ($P<0.05$) was utilized to determine significance between the responses to L-arginine and those to polyamines in Fig. 3.

Behavioral experiments

Two experiments were conducted. The first examined whether exposure to polyamines stimulated changes in individual behavior similar to that observed in response to L-amino acids, which are established feeding stimuli. The second experiment tested whether polyamines were attractive or repulsive.

Experiment 1

The first experiment observed the behavior of groups of goldfish and followed a well-established behavioral testing

protocol (Sorensen et al., 1988, 1989; DeFraipont and Sorensen, 1993). All fish were in good condition, held under a long (16 h:8 h light:dark) photoperiod and fed *ad libitum* with flake food (Chemaqua, CA, USA). Although most fish were tested only once, a small number were tested a second time with different stimuli after a 3-week intersession period, during which they were held in 1000 l stock tanks. The following odors were employed for the first experiment: well water control (i.e. a sample of the same aquarium water in which the fish were held), 10^{-2} mol l^{-1} L-serine hydrochloride [an amino acid that is a strong olfactory stimulant, but a poor tastant in goldfish (Sorensen et al., 1987; P. W. Sorensen and T. H. Hara, unpublished results)], 10^{-2} mol l^{-1} L-proline hydrochloride [a potent taste stimulus, but a poor olfactory stimulant in the goldfish (Sorensen et al., 1987; Hara, 1994)], 10^{-2} mol l^{-1} putrescine dihydrochloride, 10^{-2} mol l^{-1} spermine tetrahydrochloride, 10^{-2} mol l^{-1} cadaverine dihydrochloride and crude food odor (made by placing 40 g of flaked food into 200 ml of deionized water for approximately 1 h, and then filtering it to remove particulates). Odorants were prepared as needed and maintained at –4°C. Concentrations were chosen so that when fully diluted (1000–10 000 times, see below) and encountered by fish, they evoked approximately the same sized EOG responses.

For testing, groups of three fish were placed into 70 l glass aquaria, each of which was supplied with flowing 17°C well water (100 ml min^{-1}) and maintained on the same photoperiod as the stock tanks. Fish were allowed to adjust to these aquaria overnight (24 h.). All aquaria were shielded on three sides with a plastic screen, but had a clear front with a horizontal and a vertical line drawn to assess fish swimming rates. Gravel was used as substrate within the aquaria. An air stone was also placed in the corner of each aquarium, with a 1.5 m (i.d.=0.76 mm) length of Tygon flexible plastic tubing connecting the stone to a plastic 10 ml syringe that was used to inject the odor solutions. The syringes were positioned below the aquaria so the fish could not see them. An opaque black plastic sheet with a small viewing hole was also stretched across the front of each aquarium so that the fish could not see the observers who sat at a distance of 1–2 m. To start an experiment, each group of fish was observed for a 4 min pre-test period, after which 10 ml of test odor were then injected into the aquarium at a moment when the fish were not near the air stone. After a 15 s period to permit complete dilution of the odor (confirmed by dye tests), fish were observed for a 4 min test period. The following behaviors (from DeFraipont and Sorensen, 1993) were noted: (1) swimming activity, i.e. the total number of times that individual fish completely crossed either of the lines drawn across the front of the aquaria; (2) feeding activity, i.e. the number of times that fish rapidly opened and closed their mouths in mid-water (‘snapping’) or picked up gravel off the bottom (‘biting’; a characteristic behavior of goldfish when sampling for food on the bottom); (3) social activity, i.e. the number of times that fish physically touched each other, termed nudging behavior (DeFraipont and Sorensen, 1993). Activities were recorded as they occurred using a manual counter. All stimuli were tested 10 times on 10 different groups of fish. Because these data were ordinal and not normally distributed (Kolmogrov-Smirnoff; Instat, San Diego, CA, USA), they were analyzed using nonparametric tests. Briefly, starting (pre-test) values were compared across test groups using a Kruskall–Wallis test (Instat, San Diego, CA, USA) to confirm that they were the same. Next, for each test odor and behavior, pre- and test values were compared using a Wilcoxon matched pairs test.

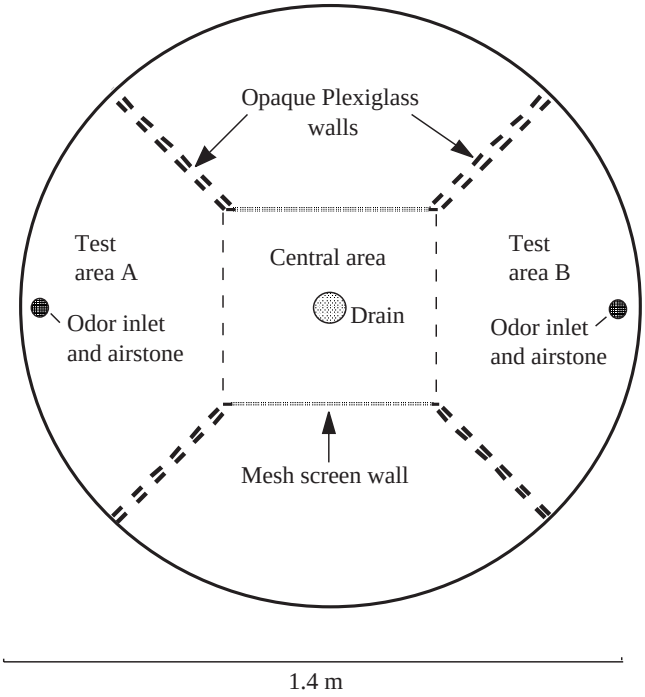

Fig. 1. Schematic diagram of the open field maze used in the attraction tests. See Materials and methods for details.

Experiment 2

The second experiment tested for attraction using a large (1.4 m diameter, 19 cm in depth, 300 l), still-water circular maze divided into two test areas and a neutral middle zone (Fig. 1). Gravel was placed on the bottom and the apparatus was lit by an overhead light on a 16 h:8 h light:dark photoperiod. The same odorants as in Experiment 1 were tested in this experiment, although they were made up and added at a slightly higher concentration (10^{-1} mol l^{-1}), because the greater size of the maze resulted in greater dilution, enabling us to add odors at times when fish were not near the stimulus port. The maze was surrounded by a dark canvas apron and had a 10 cm overhead hole through which the fish could be observed.

Test protocols followed those of Maniak et al. (2000). Groups of five fish were introduced into this maze the day before testing. The next morning, the fish were observed for a pre-test period, after which a test stimulus was introduced into the side that contained the fewer number of fish; a blank control was introduced into the other side. Each test stimulus was

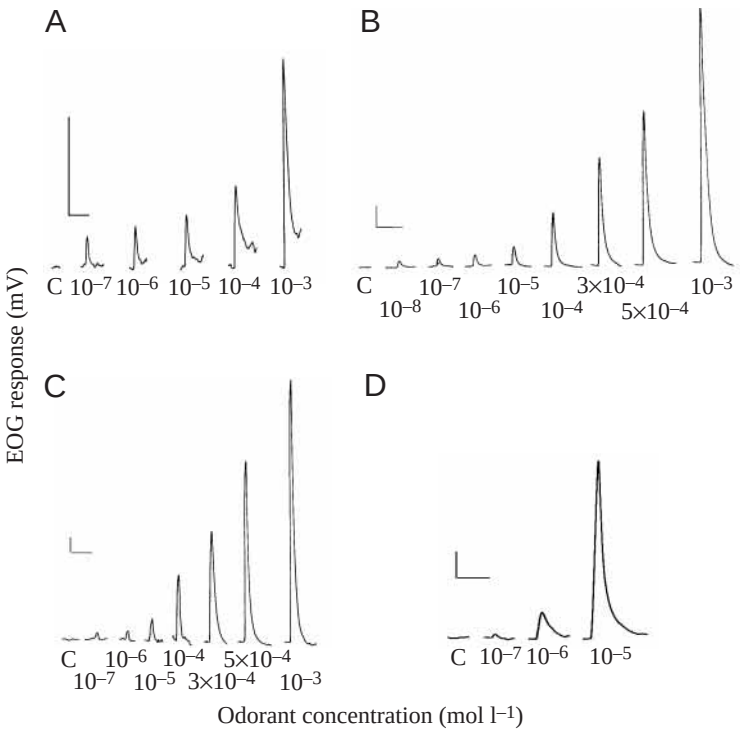

Fig. 2. Typical electro-olfactogram (EOG) recordings to ascending concentrations of (A) L-arginine, (B) putrescine, (C) cadaverine and (D) spermine, performed in a single goldfish. Odorant concentrations (in mol l^{-1}) eliciting each response are listed below each respective trace. C, charcoal filtered tapwater. Scale bars, 0.5 mV (vertical); 30 s (horizontal).

introduced as a 30 ml bolus, which was injected by a remote plastic syringe through 0.76 mm polyethylene tubing (Tygon) attached to an air stone and resulted in a final concentration of 10^{-5} mol l^{-1}. Dye tests showed that odor distribution remained restricted to the injection area for 15 min. For each trial, the fish were continuously observed during both the pre-test and test periods, and their positions were noted every minute for 12 min periods. All stimuli were tested at least seven times and the maze was drained and flushed between trails for a day. Data were analyzed using Wilcoxon matched-pairs test (Instat, San Diego, CA, USA).

Results

Electrophysiological experiments

EOG recordings to polyamines

EOG responses to putrescine, cadaverine and spermine, and the amino acid standard, 0.1 mmol l^{-1} L-arginine (Arg), were recorded to determine the relative effectiveness of polyamines as odorant stimuli. The EOG responses to polyamines were compared to that to Arg, the most potent amino acid for goldfish (Zippel et al., 1997). EOG recordings indicated that the thresholds for polyamines and Arg were similar (10^{-8}–10^{-7} mmol l^{-1}) (Figs 2, 3). At equivalent concentrations (0.1 mmol l^{-1}), putrescine, cadaverine and spermine elicited EOG responses 4.2±0.1×, 4.3±0.1× and 10.3±0.4× (mean ± S.E.M.) greater than Arg (0.4±0.2 mV; mean ± S.D.; N=10 fish), respectively (Fig. 3). Further, at supra-threshold concentrations (10^{-5}–10^{-3} mol l^{-1}), polyamines evoked a significantly larger (P<0.05; Student's t-test) EOG response than Arg (Fig. 3). Polyamines, in addition to being more potent odorants than Arg, elicited a greater magnitude of EOG response than structurally similar single amine containing compounds (amylamine and butylamine; deaminated analogs of cadaverine and putrescine, respectively) (Fig. 3).

Integrated multiunit recordings to polyamines

To determine whether (1) the EOG responses to polyamines are transduced into action potential activity of ORNs, as are amino acids (Fig. 4A), and (2) the large relative EOG effectiveness of polyamines compared to amino acids is reflected in olfactory neural (i.e. action potential) activity, integrated multiunit responses of ORNs were also recorded in a subset of eight fish. An increase in action potential activity

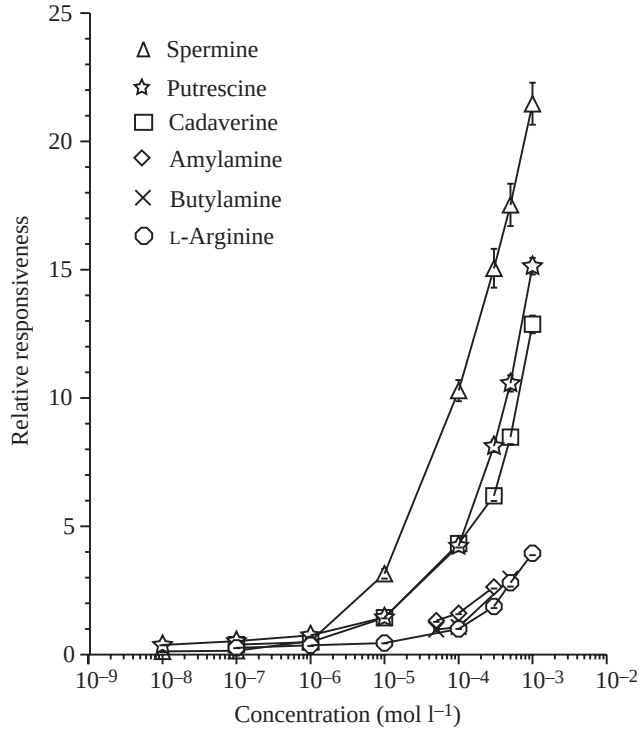

Fig. 3. Compiled electro-olfactogram (EOG) responses of goldfish to ascending concentrations of L-arginine, polyamines (putrescine, cadaverine and spermine) and single amine containing compounds (amylamine and butylamine). Responses were standardized to 0.1 mmol l^{-1} L-arginine, which evoked a response of 0.4±0.2 mV (mean ± S.D.). Values are means ± S.E.M. 3–6 fish were tested for each concentration series. Putrescine, cadaverine and spermine evoked responses significantly greater than L-arginine at all tested odorant concentrations ≥10^{-5} mol l^{-1} (Student's t-test; P<0.05).

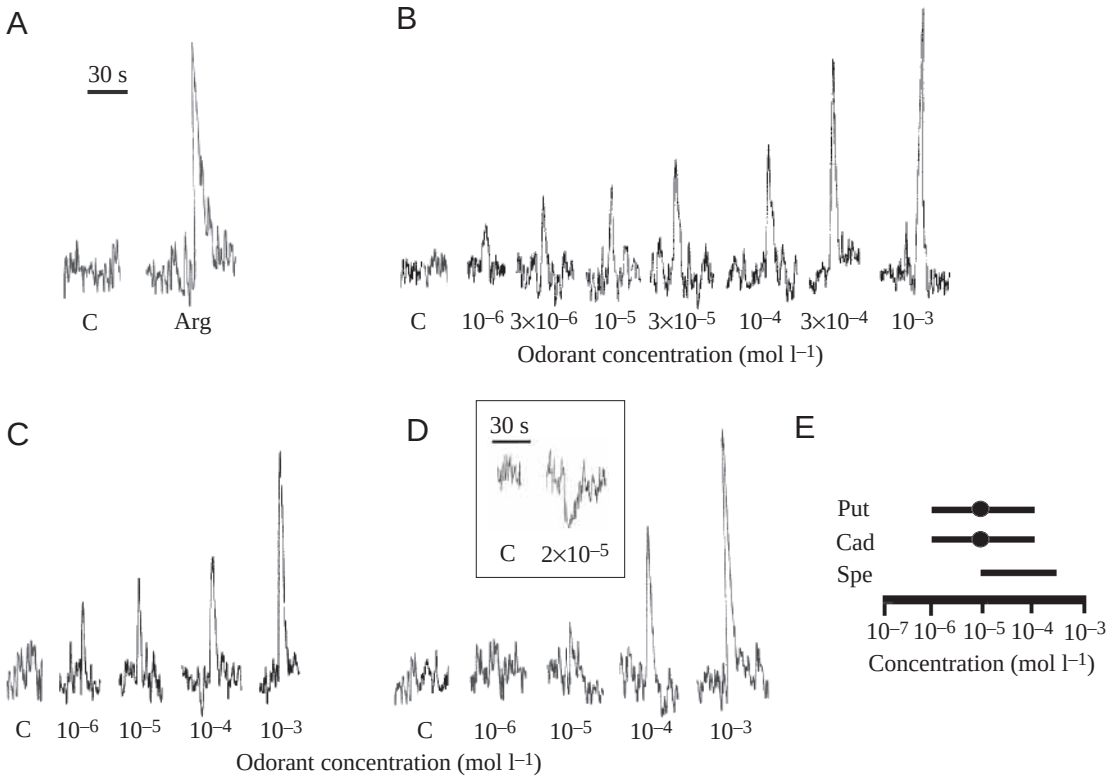

Fig. 4. Integrated multiunit recordings obtained from a single electrode location in the sensory epithelium to: (A) control (C) and 0.1 mmol l^{-1} L-arginine (Arg); (B) putrescine, (C) cadaverine and (D) spermine (inset shows an example of spermine decreasing background neural activity). (E) The chart indicates the median (filled circle) and range (horizontal bar) of estimated electrophysiological thresholds (mol l^{-1}) to putrescine (Put; *N*=5 fish), cadaverine (Cad; *N*=5) and spermine (Spe; *N*=2). Odorant concentrations (mol l^{-1}) in A–D are listed below each trace.

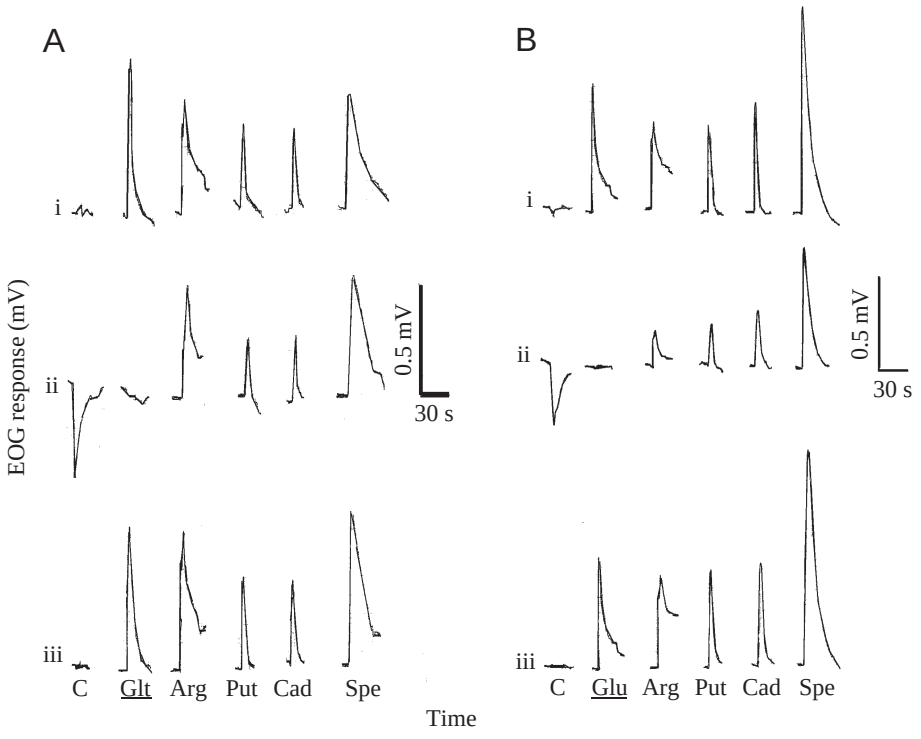

Fig. 5. Representative recordings from electro-olfactogram (EOG) cross-adaptation experiments recorded prior to (i), during (ii) and after (iii) adaptation to (A) 1 mmol l^{-1} glutaric acid (a deaminated analog of glutamate; pK_a=4.34 and 5.22) and (B) 1 mmol l^{-1} L-glutamate (a negatively charged amino acid). The adapting solution is underlined. C, CFTW control; Glt, glutaric acid; Glu, L-glutamate; Arg, 0.1 mmol l^{-1} L-arginine; Put, 10 μmol l^{-1} putrescine; Cad, 10 μmol l^{-1} cadaverine; Spe, 3 μmol l^{-1} spermine.

was evident across stimulus concentrations in olfactory responses to putrescine (in five of eight fish tested; no response in three fish), cadaverine (in five of eight fish tested; no response in three fish) and spermine [in two of eight fish tested; no response in three fish; decline in baseline activity in three fish (inset, Fig. 4D); Fig. 4B–D]. Neural thresholds (based on integrated action potential activity) further indicated that median thresholds were approximately 10^{-5} mol l^{-1} (Fig. 4E).

The potency of the polyamines with respect to Arg, the amino acid standard, was noticeably less for the higher polyamine concentrations than indicated by the EOG recordings (compare Figs 4 and 3). To investigate the discrepancy between the relative magnitude of the EOG and multiunit recordings to polyamines, the net positive charge of the polyamine molecules was examined to determine whether this charge contributed to the magnitude of the EOG responses to polyamines. The amine groups of the polyamines tested in this study have pK_a values of 8.9–11.5, which would result in a net positive charge when the pH of the polyamine test solution was adjusted to match the CFTW (pH 8.7) bathing the olfactory mucosa. To negate this excess positive charge of the polyamine molecules, the olfactory organ was adapted to 1 mmol l^{-1} L-glutamate, a negatively charged amino acid (*N*=2 fish; pK_a=2.2, 4.3 and 9.7) and 1 mmol l^{-1} glutaric acid, a decarboxylated analog of glutamate (*N*=2 fish; pK_a=4.34 and 5.22), respectively, during EOG recordings to individual applications of Arg, 10 μmol l^{-1} putrescine, 10 μmol l^{-1} cadaverine and 3 μmol l^{-1} spermine. EOG responses to Arg and the polyamines persisted with only slight attenuation in the background of both 1 mmol l^{-1} L-glutamate and 1 mmol l^{-1} glutaric acid, respectively (Fig. 5).

Polyamines bind olfactory receptor sites that are at least partially independent from those that bind other known classes of odorants

Polyamines tested during continuous presentation of either L-amino acids, bile salts or ATP to the olfactory organ elicited responses significantly greater than those to the adapting stimuli (one-way ANOVA; Tukey's *post hoc* test, $P<0.05$), but of comparable magnitude to those of odorants representing the separate odorant classes (Figs 6A-C, 7A-C). Further, adaptation to a mixture of polyamines did not significantly attenuate the response to mixtures of L-amino acids, bile salts or ATP to control levels (one-way ANOVA; Tukey's *post hoc* test, $P<0.05$) (Figs 6D, 7D). The response to the adapting stimulus, however, was reduced to control level.

Adaptation to spermine alone, but not to putrescine or cadaverine, resulted in partial cross-reactivity with L-arginine, L-lysine and L-ornithine, reducing the magnitude of the response to these compounds by 38–49% of their unadapted responses (Fig. 8C); however, responses to the tested amino acids remained significantly greater than the response to the adapting solution (one-way ANOVA; Tukey's *post hoc* test, $P<0.05$).

Multiple olfactory receptor site types for polyamines

To determine if putrescine, cadaverine and spermine bind to

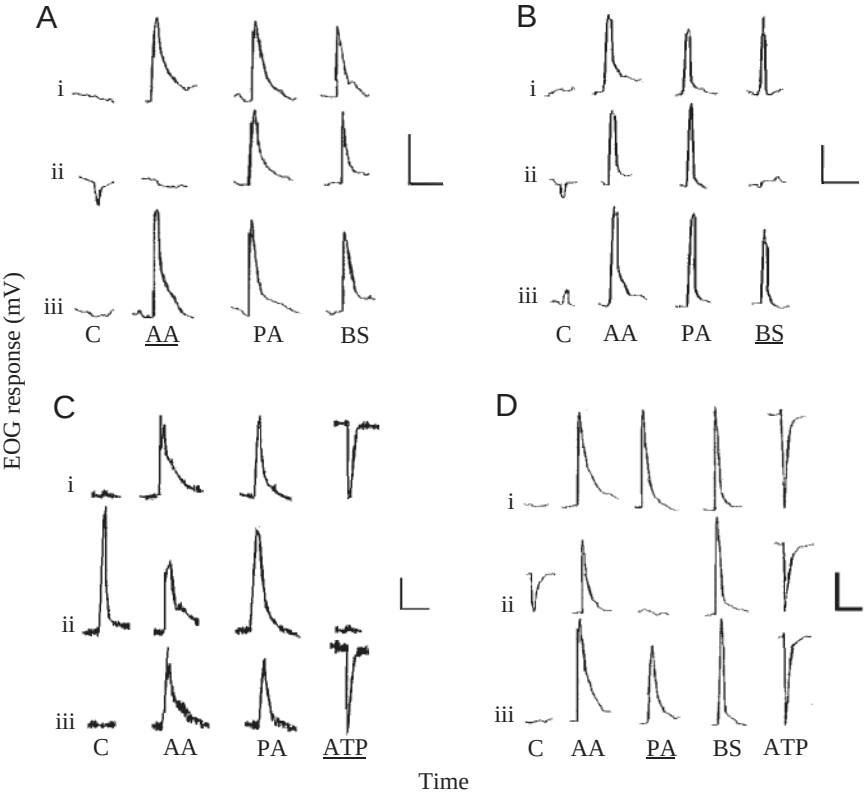

Fig. 6. Representative recordings of electro-olfactogram (EOG) cross-adaptation experiments prior to (i), during (ii) and after (iii) adaptation to: (A) a mixture of 0.1 mmol l^{-1} L-amino acids (AA; alanine, arginine, glutamate and methionine), (B) a mixture of 0.3 μmol l^{-1} bile salts (BS; sodium taurocholate and taurolithocholate), (C) 40 μmol l^{-1} ATP (compounds containing phosphate groups result in positive EOG deflections in goldfish), and (D) a mixture of polyamines (PA; 10 μmol l^{-1} putrescine and cadaverine and 3 μmol l^{-1} spermine). The adapting solution is underlined. The concentrations of the test solutions varied: in A, PA = 20 μmol l^{-1} putrescine, cadaverine and 2 μmol l^{-1} spermine) and BS = 10 μmol l^{-1} TCA and TCLA; in B, AA = 10 μmol l^{-1} alanine, arginine, glutamate and methionine and PA = 3 μmol l^{-1} putrescine, cadaverine and 1 μmol l^{-1} spermine; in C, AA = 100 μmol l^{-1} arginine, methionine, alanine and glutamate and PA = 10 μmol l^{-1} putrescine, cadaverine and 2 μmol l^{-1} spermine; in D, AA = 100 μmol l^{-1} alanine, arginine, glutamate and methionine and BS = 5 μmol l^{-1} TCA and TCLA; ATP = 40 μmol l^{-1}. Upward deflections from the baseline are negative. Responses that are positive (downward) deflections were offset to enable comparison. Scale bars, 0.5 mV (vertical); 30 s (horizontal).

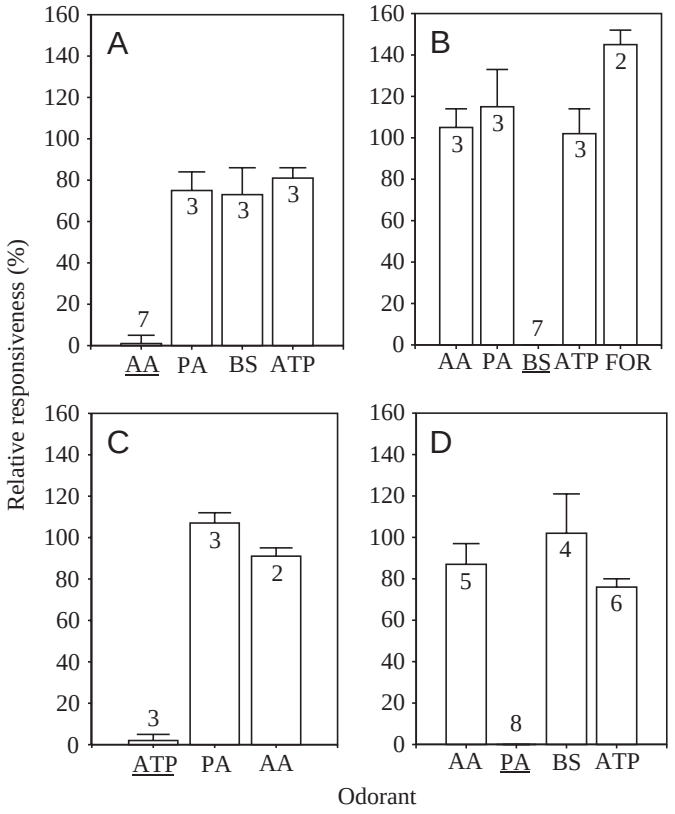

Fig. 7. Results of cross-adaptation experiments utilizing mixtures of odorants. Adaptation to (A) L-amino acids (alanine, arginine, glutamate and methionine; 10–500 μmol l^{-1}), (B) bile salts (sodium taurocholate and taurolithocholate; 0.3–10 μmol l^{-1}), (C) ATP (30–70 μmol l^{-1}) and (D) polyamines (1–40 μmol l^{-1} putrescine, cadaverine and spermine). The adapting solution in A–D is underlined. Bars indicate the percentage of unadapted response (mean ± S.D.). Numbers associated with each bar indicate the number of fish tested for each odorant category. Responses to test stimuli (A–D) are all significantly greater than the response to the adapting solution (one-way ANOVA, Tukey's *post hoc* test, $P<0.05$).

a single generic type of polyamine receptor or to different types of polyamine receptors, EOG responses to each polyamine were recorded during adaptation to other individual polyamines. During adaptation to each of the three tested polyamines, EOG responses to the remaining two test polyamines were significantly greater than that to the adapting stimulus, ranging from 42% to 72% of their unadapted responses (one-way ANOVA; Tukey's *post hoc* test, $P<0.05$) (Fig. 8A–C). Responses to the adapting polyamine in the three paradigms were reduced to the control level.

Polyamine olfactory receptors are independent from olfactory amine receptors

To further investigate the independence of polyamine receptor sites, related single amine containing compounds, the deaminated analogs of cadaverine and putrescine, amylamine and butylamine were tested during adaptation to individual polyamines. The single amine containing compounds elicited responses that ranged in magnitude from 68% to 79% of the unadapted response during polyamine adaptation (Fig. 8A–C). Adaptation to any one of the three polyamines did not significantly attenuate the EOG response to either amylamine or butylamine to control levels, suggesting that polyamine receptors do not bind single amine compounds with high affinity (one-way ANOVA; Tukey's *post hoc* test, $P<0.05$). In reciprocal experiments during amylamine or butylamine adaptation performed in a single fish, EOG responses to the individual polyamines were not eliminated and remained at 42–58% of the unadapted response (data not shown).

Effects of forskolin on odor evoked responses

To determine if transduction of polyamine odorant information and that for other known odorant classes in teleosts involve the cAMP second messenger pathway, forskolin (an adenylate cyclase activator) was continuously applied to the olfactory mucosa on the assumption that the forskolin treatment would either decrease the number of adenylate cyclase molecules available for G-protein coupled receptor activation or desensitize certain components of this pathway (e.g. cyclic nucleotide gated channels or the odorant receptors), resulting in an attenuation of the response to odorants utilizing this pathway. Odor-evoked responses to polyamines were slightly attenuated by the forskolin treatment, but not to control levels (one-way ANOVA; Tukey's *post hoc* test, $P<0.05$). During adaptation to forskolin (5-20 μmol l^{-1}), however, the magnitude of the EOG response to a mixture of bile salts (TCA and TLCA) was reduced to baseline levels, while the magnitude of the EOG responses to ATP remained relatively unaffected. During the forskolin treatment, the response to a mixture of L-amino acids (alanine, arginine, glutamate and methionine) was reduced to 59±12% (mean ± S.D.) (Fig. 9A,C). Importantly, adaptation to the mixture of bile salts did not attenuate the response to forskolin (Fig. 7B). This non-reciprocal cross-adaptation between forskolin and bile salts indicates that forskolin, whose structure resembles that of bile salts, did not compete for the bile salt receptors. Consistent with these data, EOG responses to bile salts were only slightly attenuated during continuous application of 20 μmol l^{-1} 1,9-dideoxyforskolin (an inactive analog of forskolin), which is equivalent to the highest concentration of forskolin tested ($N=2$ fish; Fig. 9B).

Effects of U73122 and U73343 on odor evoked responses

To determine if transduction of polyamine odorant information and that for other known odorant classes in teleosts involve the IP_3 second messenger pathway, U-73122 [a potent inhibitor of agonist-induced phospholipase C (PLC) activation] was continuously applied to the olfactory mucosa. The assumption was that U-73122 treatment would inhibit G-protein coupling with PLC resulting in an attenuation of the response to odorants utilizing this pathway. U-73122 (1 μmol l^{-1}) did not elicit an appreciable EOG response (<0.09 mV) when applied to the olfactory mucosa; therefore, the concentrations of the odorant stimuli tested were equivalent

to those used during the forskolin treatment. During adaptation to 1 μmol l^{-1} U-73122 (*N*=3 fish), the magnitudes of the EOG response to a mixture of polyamines, bile salts and to ATP were relatively unaffected (Fig. 10A,B). However, responses to a mixture of L-amino acids were reduced to 57±12% (one-way ANOVA; Tukey's *post hoc* test, $P<0.05$). By contrast, during adaptation to1 μmol l^{-1} U-73343 (a weak inhibitor of agonist-induced PLC activation) (*N*=3 fish), the magnitude of the EOG response to the test stimuli were not affected significantly (Fig. 10C,D).

Behavioral experiments

Exposure to crude food odor extract stimulated large (approximately tenfold) increases in feeding activity ($P<0.01$) as well as an approximate doubling of swimming activity ($P<0.01$; Fig. 11A). In contrast, adding well water alone to tanks was without any apparent effect on any behavior, as was the preovulatory sex pheromone. Exposure to spermine, putrescine, and cadaverine elicited approximately threefold increases in feeding activity ($P<0.01$), but had no effect on swimming or nudging (data not shown). Similar increases in feeding behavior were elicited by exposure to the three amino acids, L-serine, L-proline and L-arginine ($P<0.01$).

The second behavior experiment found spermine and food odor to be highly attractive to groups of goldfish ($P<0.01$) (Fig. 11B). L-serine and putrescine were also attractive ($P<0.05$), while neither blank water control nor L-proline had any effect on fish distribution.

Discussion

Polyamines are essential, naturally occurring compounds

Polyamines (putrescine, cadaverine and spermine) are naturally occurring aliphatic polycations, widely distributed in biological materials and serving a host of cellular functions (Tabor and Tabor, 1984). Intracellular concentrations of polyamines have been reported in the millimolar range for most tissues and organisms, but concentrations vary with cell type, health and growth cycles (Tabor and Tabor, 1976, 1984; Ortiz et al., 1983). Increasing concentrations of putrescine, cadaverine and spermine appeared to be correlated with the degree of decomposition of certain aquatic animals (Mietz and Karmas, 1978) and are therefore probably prevalent in the aquatic environment where they are presumably encountered by teleosts. The current study provides electrophysiological and behavioral evidence indicating that putrescine, cadaverine and spermine are odorants to goldfish, that they represent a

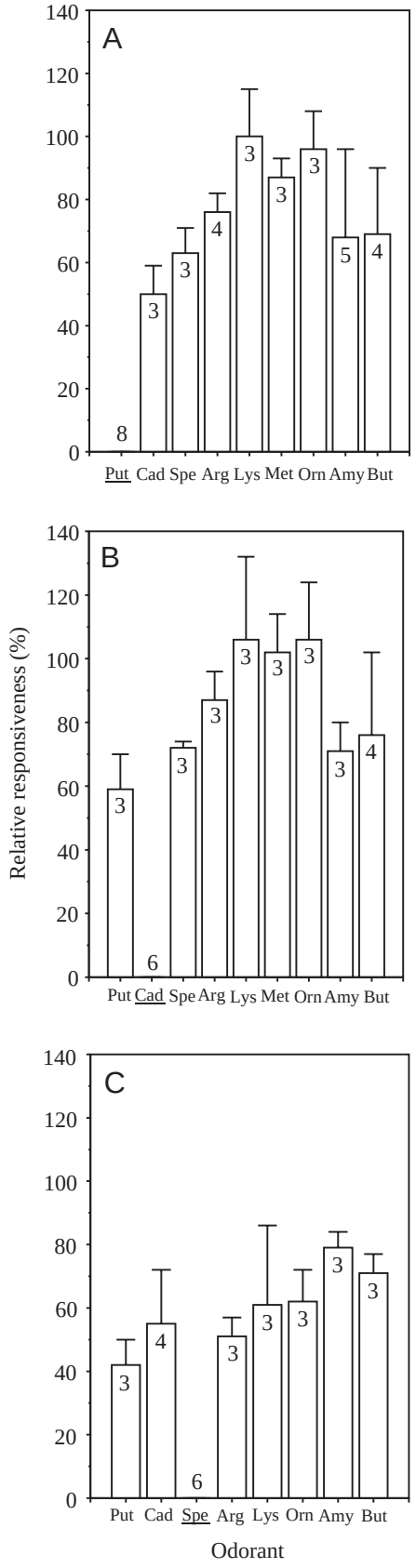

Fig. 8. Results of cross-adaptation experiments to (A) putrescine (Put; 9–50 μmol l^{-1}), (B) cadaverine (Cad; 20–40 μmol l^{-1}) or (C) spermine (Spe; 2–7 μmol l^{-1}). The adapting solution in A–C is underlined. Bars indicate percentage of unadapted response (mean ± S.D.). Numbers associated with each bar indicate the number of fish tested for each odorant. L-arginine (Arg; 0.1–1 mmol l^{-1}); L-lysine, (Lys; 0.1–1 mmol l^{-1}); L-methionine (Met; 0.1–0.5 mmol l^{-1}); L-ornithine (Orn; 0.1–1 mmol l^{-1}); amylamine (Amy; 0.03–0.5 mmol l^{-1}); butylamine (But; 0.05–0.5 mmol l^{-1}). Response magnitudes to test stimuli (A–C) are significantly greater than the response magnitude to the adapting solution (one-way ANOVA; Tukey's *post hoc* test, $P<0.05$).

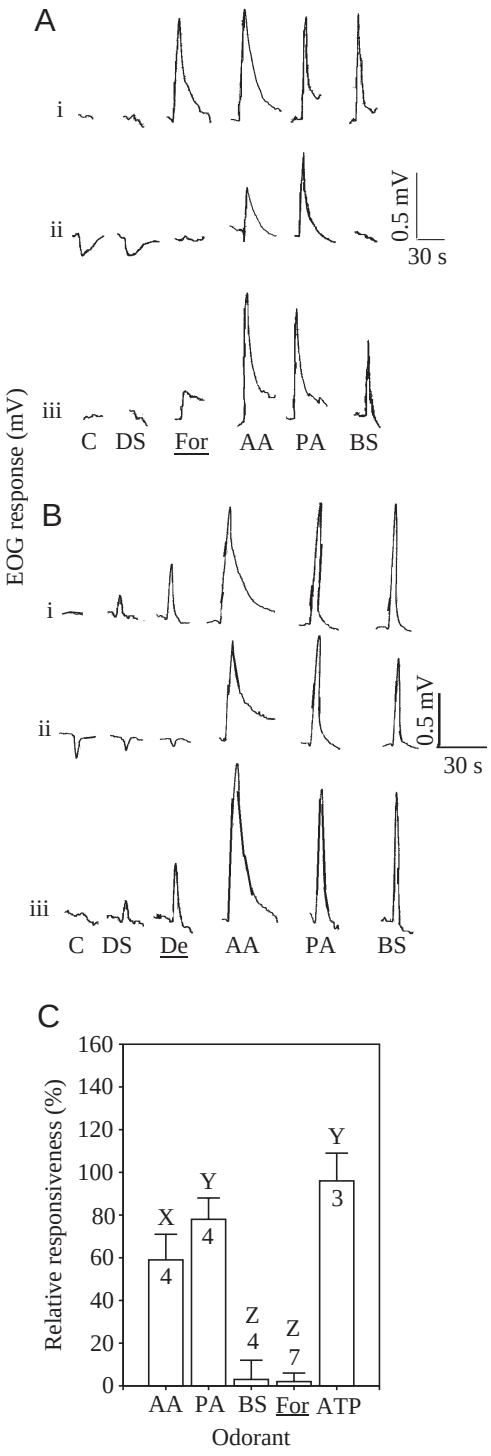

Fig. 9. The effects of forskolin and 1,9-dideoxyforskolin on odorant-evoked responses. (A) Representative electro-olfactogram (EOG) recordings to a mixture of 100 μmol l^{-1} L-amino acids (AA; alanine, arginine, glutamate and methionine), polyamines (PA; 20 μmol l^{-1} putrescine, cadaverine and 2 μmol l^{-1} spermine) and 10 μmol l^{-1} bile salts (BS; sodium taurocholate and taurolithocholate) prior to (i), during (ii) and after (iii) adaptation to 7 μmol l^{-1} forskolin (For). DS, dimethyl sulfoxide; C, CFTW control). (B) Representative EOG recordings to 100 μmol l^{-1} AA, 1 μmol l^{-1} BS and PA (10 μmol l^{-1} putrescine, cadaverine and 1 μmol l^{-1} spermine) during adaptation to 20 μmol l^{-1} 1,9-dideoxyforskolin (De). (C) Percentage of unadapted response (mean ± S.D.) to mixtures of L-amino acids (50–500 μmol l^{-1}), polyamines (1–20 μmol l^{-1}), bile salts (10–50 μmol l^{-1}), forskolin control and ATP (30–40 μmol l^{-1}) during adaptation to forskolin (5–20 μmol l^{-1}). Numbers associated with each bar indicate the number of fish tested for each odorant. The adapting solution is underlined. X, Y and Z designate statistical significance across groups (one-way ANOVA; Tukey's *post hoc* test, $P<0.05$).

class of odorants distinct from previously identified classes of odorants, and are probably primarily transduced by a non cAMP/IP_3 signaling pathway.

Peripheral recordings to polyamines

EOG dose–response recordings indicated that polyamines at concentrations of 1 μmol l^{-1} to 1 mmol l^{-1} were considerably more effective odorants for goldfish than L-arginine, the most stimulatory amino acid for goldfish. Integrated multiunit neural recordings, however, did not reflect this relative magnitude difference. Polyamines at 1 mmol l^{-1} concentration elicited integrated multiunit responses that were equal to or less than the multiunit response to 0.1 mmol l^{-1} L-arginine. Also, the electrophysiological thresholds to polyamines estimated with neural recordings were variable across the fish tested. A reasonable possibility to account for these results is that the specific ORNs that responded excitedly to polyamines are sparsely dispersed across the sensory epithelium, such that the tip of the multiunit electrode (approx. 18–25 μm platinum tip; cross-sectional area approx. 250–500 μm^2) contacted fewer polyamine-responsive ORNs relative to the number of L-arginine-responsive ORNs. If this were the case, the neural activity of the fewer polyamine-responsive ORNs would have to be driven by higher concentrations of polyamines in order to generate a response with a magnitude comparable to the response generated by the more numerous L-arginine-responsive ORNs when presented with 0.1 mmol l^{-1} L-arginine. The failure of detecting calcium changes in ORN synaptic boutons of zebrafish in response to putrescine by optical imaging (Fuss and Korsching, 2001) is consistent with our hypothesis of a sparse distribution of polyamine-responsive ORNs, especially since unpublished data indicate that polyamines do evoke EOG activity in zebrafish (W. Michel, personal communication). Further, if ORNs responding to polyamines in goldfish are few in number, and EOG responses to polyamines are of a vastly greater magnitude than those to the amino acid standard, 0.1 mmol l^{-1} L-arginine, then the transduction currents associated with individual polyamine responsive neurons are unusually large.

Polyamine olfactory receptors are independent from those for known odorant classes and related compounds

The previously identified, biologically relevant odorants to teleosts (amino acids, bile salts, nucleotides, sex steroids and prostaglandins) are initially recognized and discriminated by

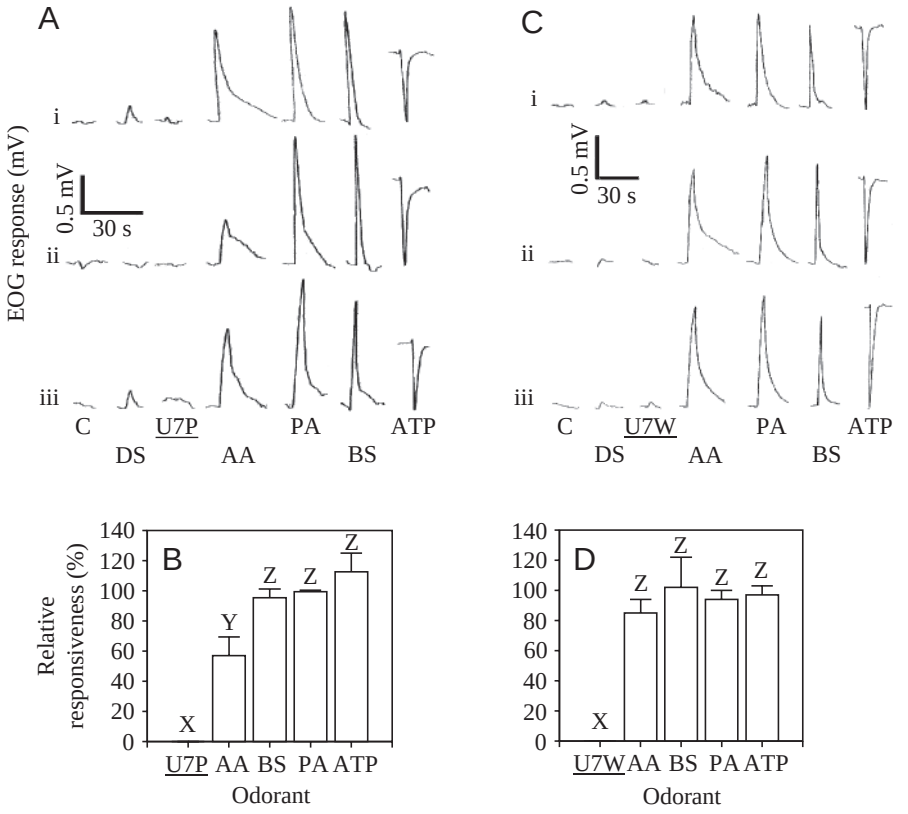

Fig. 10. The effects of the potent inhibitor of agonist-induced phospholipase C (PLC) activity, U-73122 (U7P), and its weaker analog, U-73343 (U7W) on odorant-evoked responses. (A,C) Representative electro-olfactogram (EOG) recordings to a mixture of 100 μmol l^{-1} L-amino acids (AA; alanine, arginine, glutamate and methionine), polyamines (PA; 10 μmol l^{-1} putrescine and cadaverine and 2 μmol l^{-1} spermine) and 10 μmol l^{-1} bile salts (BS; sodium taurocholate and taurolithocholate) prior to (i), during (ii) and after (iii) adaptation to 1 μmol l^{-1} U-73122 (U7P) and 1 μmol l^{-1} U-73343 (U7W), respectively. DS, dimethyl sulfoxide; C, CFTW control. The EOG response to ATP has been offset to allow for comparison. (B,D) Percentage of unadapted response (mean ± S.D.) to the adapting controls (underlined), (U7P in B; U7W in D), L-amino acids, polyamines, bile salts and ATP during adaptation to U7P (in B) and U7W (in D). Data in B and D were compiled from three fish. The adapting solution is underlined. X, Y and Z designate statistical significance across groups (one-way ANOVA; Tukey's *post hoc* test, $P<0.05$).

different molecular olfactory receptors (Sorensen and Caprio, 1998). These odorants bind to seven transmembrane domain G-protein coupled receptors located within ciliary and/or microvillar membranes of ORNs (Buck and Axel, 1991; Cao et al., 1998; Speca et al., 1999; Mombaerts, 1999). The present cross-adaptation experiments suggest that olfactory receptor sites for polyamines are relatively independent from olfactory receptor binding sites for these other known classes of odorant stimuli.

In the present experiments, EOG responses to a mixture of polyamines were not attenuated significantly by adaptation to L-amino acids, bile salts or ATP and, conversely, adaptation to a mixture of polyamines did not attenuate significantly the EOG responses to these other classes of odorants. Further, during adaptation to cadaverine and putrescine, responses to the amino acids L-lysine and L-ornithine remained unaffected. Thus, the addition of an α-carboxylic acid group to putrescine and cadaverine (resulting in L-ornithine and L-lysine, respectively) results in the binding of these compounds to different (i.e. amino acid) receptor sites. Consistent with these data, putrescine and cadaverine were shown to bind with low affinity to the L-arginine/L-lysine amino acid olfactory receptor in goldfish (Speca et al., 1999). Further, EOG responses to single amine containing compounds in the present study were not eliminated during adaptation to polyamines, nor were responses to polyamines eliminated during adaptation to amines. These data suggest that the removal of a single amine group from putrescine and cadaverine (resulting in butylamine and amylamine, respectively) decreases the affinity of these molecules for polyamine receptors. Therefore, the persistence of the EOG responses to single amine containing compounds suggests that goldfish probably possess molecular olfactory receptors with the ability to discriminate polyamines from single amine containing compounds. Single amine compounds were previously shown to be olfactory stimuli for sharks (Hodgson and Mathewson, 1978), but a recent study failed to visualize calcium influx into ORN synaptic terminals in the zebrafish olfactory bulb in response to amines (Fuss and Korsching, 2001). Although the present study suggests that the teleost olfactory system responds to single amines, future studies should reinvestigate single amine compounds as olfactory stimuli for fish.

Previous investigations in teleosts indicated that ORNs possessing receptors for different classes of compounds project axons to specific sub-regions of the olfactory bulb (Hara and Zhang, 1996; Nikonov and Caprio, 2001; Friedrich and Korsching, 1997, 1998). An independence of olfactory receptor sites for polyamines, distinct from other known classes of biologically relevant stimuli, suggests the possibility for differential processing of polyamine odorant information within the olfactory bulb of the goldfish and possibly for other teleost species. In addition, the identification of independent olfactory receptors for polyamines may possibly aid research efforts into the molecular and biochemical characterization of teleost orphan olfactory receptors.

Relatively independent receptor sites for different polyamines

In addition to receptor sites for polyamines being independent of those for other known biologically relevant

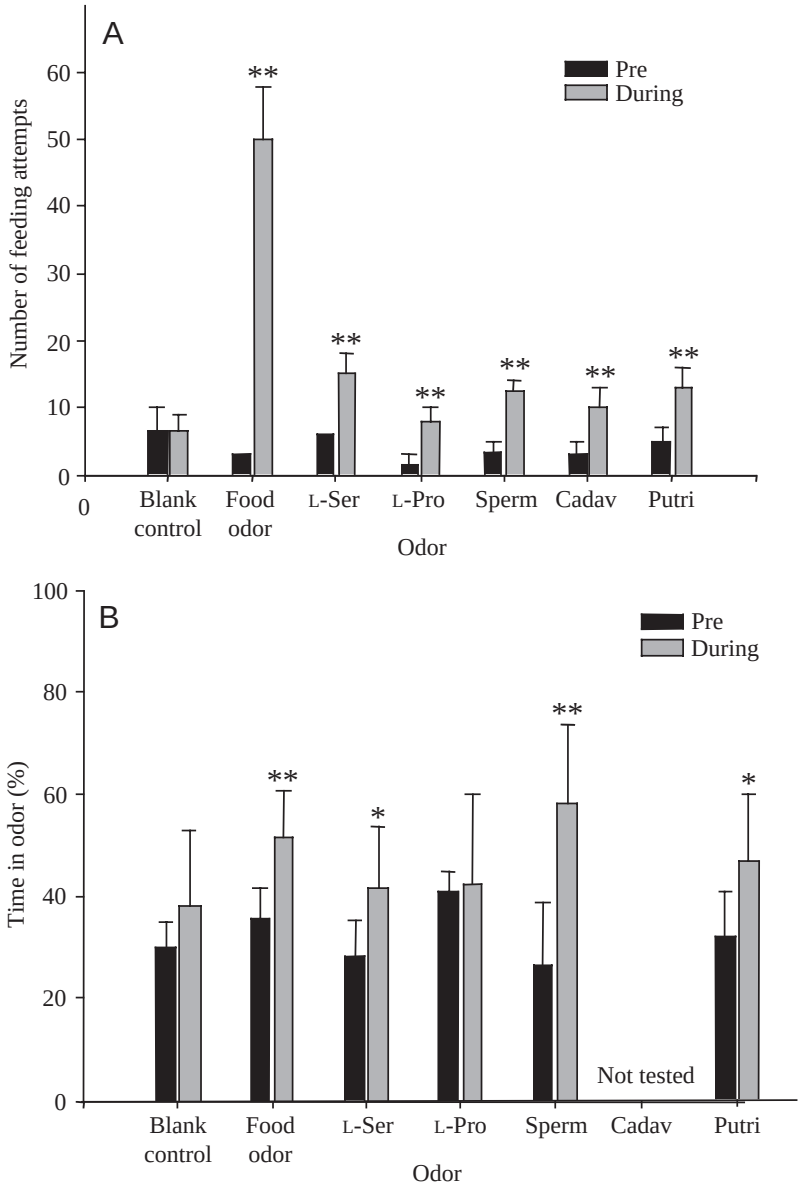

Fig. 11. (A) Results of Behavior Experiment 1. The median (and 75% quartile) number of snapping and biting behaviors exhibited by groups of goldfish in a 4 min period before odor was added ('Pre') and then while odor was present ('During') in Experiment 1. Significant differences between the Pre- and test periods are noted. $^{**}P<0.01$. (B) Results of Behavior Experiment 2. The median percentage time (75% quartile) spent by goldfish in the area of the maze to which test odors were added. Time spent during the 12 min period prior to odor addition ('Pre') and then after it had been added and was still present ('During') are shown for each stimulus. Significant differences between Pre- and test periods are noted. $^{*}P<0.05$, $^{**}P<0.01$.

odorants for fish, the present results indicate the relatively independent olfactory receptor sites among the polyamines themselves. Although the partial reduction of the EOG response to other polyamines during adaptation to a single polyamine suggests that some receptor sites possibly accommodate a number of polyamines, the cross-adaptation results indicated the existence of polyamine receptor sites that are specific for each of the different tested polyamines. These results suggest the possibility that goldfish can behaviorally discriminate among the polyamines; however, further behavioral testing is required to assess this suggestion.

Second messenger pathways in polyamine odorant transduction

Subsequent to receptor activation, cytosolic second messengers (cAMP and/or IP_3) increase *via* heterotrimeric G-protein modulation of enzymatic activity resulting in odorant-induced sensory transduction currents (Bruch, 1996; Schild and Restrepo, 1998). The present results showed that the response to polyamines persisted with only a slight reduction from control levels during forskolin adaptation, suggesting that polyamine transduction is relatively independent of the cAMP second messenger pathway. The current study also found no evidence linking the IP_3 pathway to polyamine odorant transduction. Continuous application of U-73122 (an agonist-induced PLC inhibitor) to the olfactory mucosa failed to affect the EOG response to polyamines (or to bile salts or ATP), while reducing the EOG response to L-amino acids to 57% of the unadapted response. That the IP_3 signaling cascade is involved in the transduction of L-amino acid odorant information in teleosts is also consistent with molecular investigations (Bruch, 1996).

In contrast to polyamines, the EOG response to bile salts was reduced by forskolin to control levels, while responses to L-amino acids were attenuated by approx. 41%. These data suggest that the cAMP pathway is utilized by ORNs responding to bile salt odorants, while at least some ORNs responding to L-amino acids also utilize this pathway. The forskolin results are consistent with data from similar experiments obtained in zebrafish (Michel, 1999); however, species differences may also occur as the IP_3 pathway was reported to be involved in the transduction of bile salt odorant information in the Atlantic salmon *Salmo salar* (Lo et al., 1994).

The elimination of ORN responses to bile salts by forskolin might have occurred *via* multiple mechanisms. Direct activation of adenylate cyclase by forskolin could have effectively saturated enzymatic activity, reducing the number of enzymes available for receptor activation. Alternatively, protein kinase A activation by elevated cAMP concentrations could have desensitized the bile salt receptors (Boekhoff and Breer, 1992; Schleicher et al., 1993). Also, increased cytosolic cAMP concentrations could have opened cyclic nucleotide-gated channels, elevating intracellular calcium concentrations

and leading to increased channel susceptibility to intracellular calcium block (Frings et al., 1992; Balasubramanian, 1996) and decreased affinity of the cyclic nucleotide-gated channel for cAMP (Kramer and Siegelbaum, 1992). It is also possible that forskolin might have competed for bile salt receptors; however, continuous application of bile salts to the olfactory mucosa did not reduce the EOG response to forskolin. In addition, continuous application of 1,9-dideoxyforskolin (an inactive analog of forskolin) did not attenuate the EOG response to bile salts. Irrespective of which of these possible mechanisms were operating, our collective data suggest that bile salt odorant information is transduced in goldfish *via* the cAMP second messenger pathway.

Spermine attenuation of responses to amino acids and spontaneous activity

Although the polyamines spermine, cadaverine and putrescine were each used as adapting stimuli in the present study, only spermine as an adapting stimulus attenuated EOG responses to amino acids; also, the response to spermine in some multiunit preparations recorded with the microelectrode only caused a reduction in baseline spontaneous activity. Possibly both of these effects were a direct result of spermine block of specific ORN ion channels. Polyamines have been indicated to modulate a variety of ion channels, including K_{ir} channels (Fakler et al., 1994; Lopatin et al., 1994; Ficker et al., 1994; Pellegrini-Giampietro, 2003), glutamate receptor channels (Bowie and Mayer, 1995; Donevan and Rogawski, 1995; Kamboj et al., 1995), voltage-gated calcium and potassium channels (Droiun and Hermann, 1994), K_{ATP} channels (Niu and Meech, 1998), calcium-activated potassium channels (Weiger et al., 1998), nAchR channels (Haghighi and Cooper, 1998) and retinal rod cyclic nucleotide-gated channels (Lu and Ding, 1999). Intracellular putrescine and intra- and extracellular spermine were also indicated to attenuate the conductance of the rat olfactory cyclic nucleotide-gated (CNG) channel (Lynch, 1999; Nevin et al., 2000). Consistent with the possibility that spermine blocks CNG channels of ORNs, forskolin experiments in zebrafish (Michel, 1999) and goldfish (present study) indicated that the cAMP signaling pathway, and therefore the CNG channel, is involved in L-amino acid odorant transduction. In the present experiments, both forskolin and spermine treatments reduced the EOG responses to L-amino acids by comparable margins, 41% and 38–49%, respectively.

Behavioral responses to polyamines

Both behavioral experiments strongly suggest that polyamines function as feeding cues in the goldfish. Both putrescine and spermine elicited high levels of spontaneous feeding behavior that were similar in nature and magnitude to the two amino acids, L-serine and L-proline. Further, no changes in social behavior or swimming rates were seen to any of these cues. It was reasonable that food odor alone was a stronger feeding stimulus than any of the synthetic cues, since naturally occurring foods are a mixture of many different compounds. Interestingly, the polyamines, and spermine in particular, were strong attractants, perhaps stronger than L-serine and equivalent to food odor itself. In contrast, L-proline, a strong tastant, but a poor odorant in this species (Hara, 1994; P. W. Sorensen and T. J. Hara, unpublished results), failed to cause the fish to spend more time within that odor than the control, suggesting that olfactory cues may have a greater role in attraction in this species than gustation, and that polyamines exert the bulk of their behavioral activity through the olfactory system.

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