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Chemosensory reception, behavioral expression, and ecological interactions at multiple trophic levels

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Summary

Chemoreception may function throughout an entire animal lifetime, with independent, stage-specific selection pressures leading to changes in physiological properties, behavioral expression, and hence, trophic interactions. When the California newt (*Taricha torosa*) metamorphoses from an entirely aquatic larva to a semi-terrestrial juvenile/adult form, its chemosensory organs undergo dramatic reorganization. The relationship between newt life-history stage and chemosensory-mediated behavior was established by comparing responses of adults (as determined here) to those of conspecific larvae (as studied previously). Bioassays were performed in mountain streams, testing responses of free-ranging adults to 13 individual L-amino acids. Relative to stream water (controls), adults turned immediately upcurrent and moved to the source of arginine, glycine or alanine release. These responses were indicative of predatory search. Arginine was the strongest attractant tested, with a response threshold (median effective dose) of 8.3×10^{-7} mol l^{-1} (uncorrected for dilution associated with chemical release and delivery). In contrast to adult behavior, arginine suppressed cannibal-avoidance and failed to evoke search reactions in larvae. For a common set of arginine analogs, the magnitudes of adult attraction and larval suppression were not positively correlated. Suppression of cannibal-avoidance behavior in larvae was unaffected by most structural modifications of the arginine molecule. Adult behavior, on the other hand, was strongly influenced by even subtle alterations in the parent compound. Reactions to arginine in both adults and larvae were eliminated by blocking the external openings of the nasal cavity.

Stimulating adult predatory search in one case and inhibiting larval cannibal avoidance in the other, arginine is a chemical signal with opposing behavioral effects and varying ecological consequences. Significant differences between responses of adults and larvae to changes in arginine structure suggest alternative, chemosensory receptor targets. Although arginine reception functions throughout an entire newt lifetime, an ontogenetic shift in larval and adult chemoreceptive ability changes behavioral expression, and thus, reflects the unique selection pressures that act at each life-history stage.

Key words: arginine, amino acid, tetrodotoxin, TTX, newt, salamander, *Taricha torosa*, predator, prey, cannibalism, chemical signal, olfaction, adult-larval interaction, odor plume, feeding, foraging, search.

Introduction

Cellular mechanisms of chemosensory reception are highly conserved across all metazoans (Ache, 1994; Hildebrand and Shepherd, 1997; Ache and Young, 2005). The same signal molecules therefore can be used in chemical communication by phylogenetically diverse species. In the ocean, for example, dimethylsulfoniopropionate (DMSP) and its metabolites [dimethylsulfide (DMS) and acrylic acid] convey information that spans multiple trophic levels and accelerates material exchange rates among bacteria (decomposers), phytoplankton (primary producers), zooplankton (secondary consumers) and seabirds (apex predators) (Nevitt et al., 1995; Zimmer-Faust et al., 1996; Wolfe, 2000; Steinke et al., 2006). Such compounds play important roles in mediating ecological interactions that structure populations and communities.

Many neurotoxins also have effects at multiple trophic levels, including acting as chemosensory stimuli for resistant consumer species (Schulz et al., 1993; Weller et al., 1999; Macel and Vrieling, 2003). The guanidine alkaloid, tetrodotoxin (TTX), for instance, serves as a chemical defense or venom for a biodiverse assemblage of animal and microbial species (Kim et al., 1975; Sheumack et al., 1978; Miyazawa et al., 1986; Kogure et al., 1996). It acts as a potent neurotoxin by binding to and blocking voltage-gated sodium channels on nerve and muscle cell membranes (Narahashi, 1994; Cestèle and Catterall, 2000). Notably, California newt (*Taricha torosa*) larvae escape cannibalism by detecting the poison (TTX), which is well-

known as a chemical defense for adult conspecifics (Zimmer et al., 2006). Following release from adult skin, TTX acts as a reliable olfactory cue, warning larvae of imminent danger. For individuals of the same newt species at different life-history stages, TTX operates in a dual role – both alerting (conspecific larval) prey and defending (adults) against predators. Stimulating a behavioral response in one case and inhibiting neural activity in the other, TTX has opposing physiological effects with strong, but contrasting, ecological consequences.

The TTX-mediated predator-avoidance reaction of newt larvae is notably absent when adults are eating alternative prey (worms) (Kerby and Kats, 1998). In this case, larval hiding behavior is suppressed by the stream-borne presence of the free-amino acid, L-arginine (Ferrer and Zimmer, 2007). Arginine is abundant in the body fluids of worms, and released upon injury into stream water (see 'Amino acid composition of worm body fluids' in the Results). From previous studies, both TTX and arginine bind receptors through charged interactions with their terminal guanidinium moieties [TTX (see Kao, 1986; Hille, 2001); arginine (see Bryant et al., 1989; Kalinoski et al., 1989; Lipsitch and Michel, 1999)]. Suppression might therefore arise from competitive interactions between these two molecules for common receptor binding sites. Presumably, the presence of dissolved arginine indicates a reduction in cannibalism risk for larvae, as a consequence of adult feeding preferences. Although a generalist forager (Stebbins, 1972; Hanson et al., 1994), adult *Taricha torosa* prefer worms over conspecific young (Kerby and Kats, 1998).

The suppressant effect of arginine on newt larval behavior is uncharacteristic as this molecule is best known as a feeding stimulant/attractant for many species of aquatic and terrestrial predators (Caprio and Byrd, 1984; Zielinski and Hara, 1988; Kang and Caprio, 1997; Carr et al., 1996; Tabuchi et al., 1996). Similarly, adult newts are voracious carnivores and eat a wide variety of invertebrate prey, including insects, snails and isopods, in addition to worms and conspecific young (Stebbins, 1972; Hanson et al., 1994). Consequently, arginine might act as an adult feeding attractant rather than a suppressant. The present investigation was performed using field experiments that quantified the behavioral responses of free-ranging adult newts to arginine and 12 other L-amino acids. Results show an ontogenetic shift in adult/larval chemosensory reception that changes the expression of arginine-mediated behavior and determines the fates of trophic interactions. This is the second of two companion manuscripts describing the behavioral mechanisms of chemoreception as a consequence of life history stage in the California newt. The present paper is written from the perspective of an adult predator, with comparisons to the larval stage. By contrast, the accompanying paper is written from the perspective of the larval prey (Ferrer and Zimmer, 2007).

Materials and methods

Amino acid analysis of worm body fluids

Amino acids are abundant in tissues of marine and freshwater invertebrates (Sutcliffe, 1962; Firling, 1977; Edwards, 1982; Herzog and Liappis, 1987; Carr et al., 1996; Zimmer et al., 1999), and they act as feeding stimulants/attractants for diverse predatory species (Carter and Steele, 1982; Carr, 1988; Valentinčič and Captrio, 1994; Coman et al., 1996; Hara, 2006; Keiichiro et al., 2006). In the present study, worm (*Eisenia rosea* Gates 1942) prey of adult newts (*Taricha torosa* Rathke 1883) were collected on three occasions (April 14, August 26 and October 12, 2004) from natural streamside sediments at the field study site. On each date, 20 worms were held in plastic specimen jars containing moist sediment during transit to the laboratory. In the lab, worms were rinsed thoroughly with organic-compound-free artificial stream water (in mmol l^{-1}: NaCl, 3.0; KCl, 0.2; $CaCl_2$, 0.2; Hepes, 1.0) to remove all sediment, and starved for 48 h to void gut contents. They were weighed (wet mass) and sliced (each with a single cut), then fluid contents were extruded into a vial containing 1 ml artificial freshwater. The resulting solutions were centrifuged (10 000 g), clarified further by filtration (0.2 μm pore size), and samples frozen immediately at –80°C.

Each filtrate was subsequently analyzed in triplicate using high-performance liquid chromatography (HPLC; Beckman System Gold v. 8.10, 126 binary Solvent Module, and 507 Autosampler; Beckman-Coulter, Inc., Fullerton, California, USA). After pre-column derivatization of amino acids with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate, 20-μl aliquots were injected onto a reversed-phase column (35°C, silica base bonded C_{18} Nova-Pak column, 4-μm particle size, 60 Å pore size, 3.9 mm inner diameter × 150 mm; Waters, Milford, Massachusetts, USA) using modifications of AccQTag (Waters) labeling and separation chemistry (Cohen and Micheaud, 1993; Zimmer et al., 1999). Using HPLC-grade reagents, a series of gradients with increasing organic modifier, starting at 150 mmol l^{-1} sodium acetate–8 mmol l^{-1} triethylamine–6 μmol l^{-1} ethylene-diamine-tetraacetic acid (pH adjusted to 5.83 with phosphoric acid and filtered to 0.45 μm) and ending with 60:40 acetonitrile:H_2O, eluted the amino acids at a constant flow of 1.0 ml min^{-1}. Separated amino acids were assayed spectrofluorometrically (Jasco FP-920 fluorescence detector with a 5 μl flow cell; excitation, λ=250 nm; emission, λ=395 nm; with slits measuring 18 nm and a gain of 100) at a data collection rate of 2 Hz. Identities and concentrations of the amino acids were based on retention times and peak areas, respectively, of external standards (Pierce, Rockford, Illinois, USA).

Field site and study animal

Experiments were performed along a 4 km stretch of Tuna Canyon Creek in the Santa Monica Mountains, Malibu, California, USA (34°03′40″N, 118°68′51″W) between April and November, 2004. There was no trail access to the stream, largely eliminating pedestrian traffic. All equipment and solutions were carried to the study site. This stream section included 35 pools that contained 103 adult newts (*Taricha torosa*). Individual newts were captured, measured (with Vernier calipers), and weighed (using a battery-operated scale)

on site, before immediate release. Adult mean length and mass were 7.3 cm (±0.5 cm s.d.) and 14.7 g (±1.5 g s.d.), respectively, and the vast majority (82.5%) of animals were males. Statistical analyses (Fisher exact tests) showed no significant difference between male and female responses to any test or control solution (data available upon request), so results were combined across gender.

Stream beds consisted of poorly-sorted sand, gravel and cobble, with small, submerged rocks (0.05–30 cm diam.). Qualitative visualizations, using Rhodamine WT dye, indicated complex, three-dimensional flows within pools. Flow speed and direction were determined near (<30 cm distant) free-ranging adults using an electromagnetic current meter (Model 523, Marsh-McBirney Corp., Frederick, Maryland, USA). The two-dimensional sensor (1 cm diam.) was mounted on a 1-m-long arm on top of a tripod, and programmed to record velocities (at 1 Hz, over 10 min intervals) in the along-stream and vertical directions. Overall, 107 flow records were taken during the study, with replicates for each pool collected at either a monthly or bi-monthly interval. In each recording, the flow sensor was placed 2 cm above the substrate – the elevation of a typical adult nose. Temperature, conductivity, pH, and light intensity were determined every other day to characterize adult habitats (Table 1).

Table 1. *Properties of the physical environment and concentration dilution associated with chemical stimulus input and delivery*

Variable*	Median	Percentile 10th	Percentile 90th
Pool size (surface area, m^2)	4.02	0.40	5.05
Maximum pool depth (m)	0.38	0.18	0.59
pH	8.02	7.84	8.10
Conductivity (μS)	1146	1123	1175
Temperature (°C)	17.8	17.1	18.5
Light intensity (μmol m^{-2} s^{-1})	15.8	13.2	21.1
Stimulus dilution factor ($\times 10^{-4}$)	2.62	1.13	4.22
Flow, along channel (cm s^{-1})			
Speed (u)	2.6	2.0	3.6
Fluctuating component (u')†	0.6	0.4	1.0
Flow, vertical (cm s^{-1})			
Speed (w)	0.5	0.1	1.2
Fluctuating component (w')†	0.5	0.3	0.7

Data are combined for 35 stream pools along a 4-km stretch of Tuna Canyon Creek (Malibu, California, USA), between April and November 2004.

*In each 10-min sampling period, stream water temperature, conductivity and pH, were recorded 2 cm above the bed at 0.1 Hz. Simultaneously, light intensity was measured at 0.1 Hz on the surface of the stream bed. Pool size was determined from digital images by computer analysis.

†Calculated as the standard deviation (root mean square) using repeated measurements (600 electromagnetic current meter recordings) taken over 10-min intervals.

Field bioassays

A test chemical or control solution was introduced to a free-ranging *Taricha torosa* adult in each 5 min trial. An investigator hid behind rock boulders that lined the stream, and continuously released solution through transparent polyethylene tubing (2 mm i.d.). The tubing was threaded in a hand-held 3-m length of transparent acrylic rod (5 mm i.d.). Positioned 2 cm above the stream bottom, the tip was, nominally, 30 cm upstream of a targeted adult. A constant delivery rate was achieved by using a microprocessor-controlled syringe pump (KD Scientific Inc., Holliston, Massachusetts, USA) in conjunction with a 12 V battery and voltage inverter. Each solution exited the tubing at 10 ml min^{-1} and passed through a foam-diffusing tip, thus minimizing hydrodynamic disturbances. The exit speed of 1.3 cm s^{-1} was essentially isokinetic with surrounding flow.

Dilution of the test solutions due to stimulus input and delivery from the point of origin to an adult newt was determined by fluorometric measurements (Table 1). After substituting Rhodamine WT dye (prepared at 1 g l^{-1}) for a test or control solution, fluorescence was recorded (over 5 min) in 76 replicate trials with a fluorometer (model 10-AU-005, Turner Designs, Mountainview, California, USA). Stream water was evacuated continuously (1 ml min^{-1}) through polyethylene tubing (2 mm i.d.) and a custom-built flow-through detector cell (50 μl volume). Field measurements were calibrated on site using dye standards prepared with stream water at five different concentrations.

Selection of test chemicals for field bioassays

Field experiments with adult newts, (1) tested for effects of L-amino acids, (2) generated a dose–response curve for arginine, and (3) determined the activities of arginine structural analogs. Several amino acids were examined for their relative capacity to evoke behavioral responses. Chosen compounds included aspartate and glutamate (acidic), arginine and lysine (basic), glycine and alanine (short-chain neutral), leucine and valine (nonpolar side chain), cysteine, methionine and taurine (sulfur containing), and phenylalanine and tryptophan (ring containing). Each amino acid was introduced at 10^{-5} mol l^{-1}. Arginine was also presented at 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} mol l^{-1} in a dose–response experiment. Finally, bioassays were run (at 10^{-5} mol l^{-1}) using arginine and arginine analogs having deletions, substitutions or additions to the carbon chain, or the guanidinium, carboxyl or amine functional groups (Fig. 1). Worm fluids, diluted to a final (total) arginine concentration of 10^{-5} mol l^{-1}, and stream water served as controls in the first and second experiments. Arginine (at 10^{-5} mol l^{-1}), as a control, was substituted for worm fluids in experiment 3. Each test or control solution was bioasssayed on 10–15 different adults.

To minimize the likelihood of retesting the same solution on a given individual, each compound (and concentration) was presented only to one adult within a particular pool. Previous research has shown that, over months to years, individual adults exhibit strong site fidelity to a specific pool (Watters and Kats,

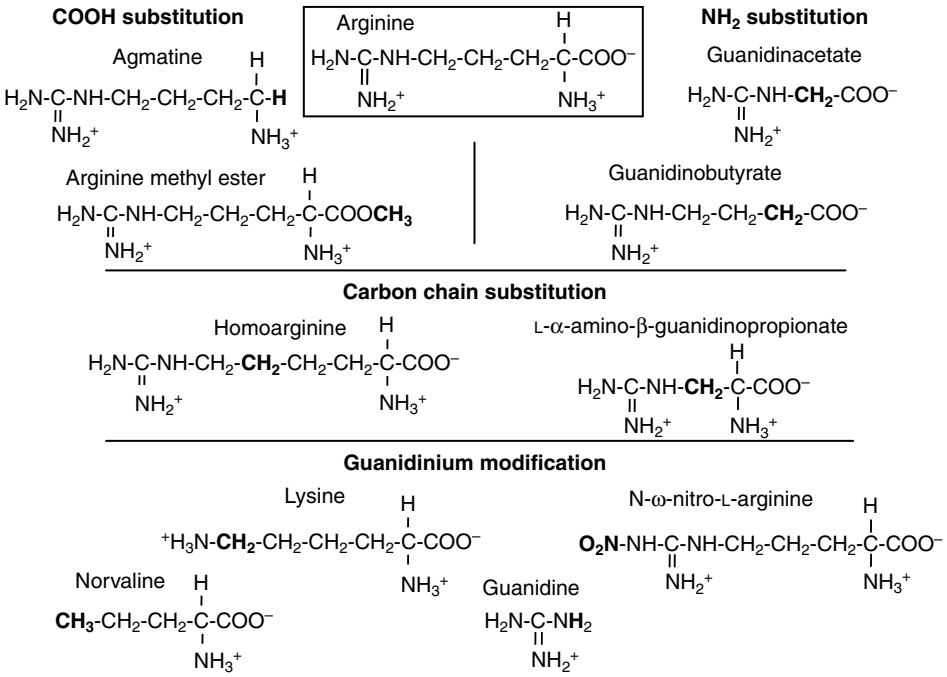

Fig. 1. Structural formulae of arginine analogues, as tested in this study.

2006). Otherwise, the order of presentation across all test or control treatments for each trial was determined, within a given experiment, using a random numbers generator. All solutions were prepared with Nanopure ddH_2O at a stock concentration of 10^{-2} mol l^{-1} in the laboratory, and then stored at –80°C. They were transported to the field on dry ice, thawed immediately prior to use (<5 min), and diluted rapidly to the desired concentrations in stream water.

Determining the role of nasal chemoreception in mediating behavioral response

A field experiment was used to determine the role of nasal chemoreception in mediating adult behavioral responses. Specifically, 30 newts were captured by hand (sterile latex gloves). Each individual was drawn from a different stream pool. The nasal cavities were then blocked by applying inert silicon gel (0.1 ml) to the external openings, or nares, with a sterile cotton swab. These newts were released back into their home pools and given 30 min to recover, during which they were observed to swim near the bottom with no apparent ill effect. Next, each individual was tested, using procedures described above, with either 10^{-5} mol l^{-1} arginine or stream water (control). Fifteen individuals were tested with each solution. To control for animal handling, a second group of 30 adults was tested in the same manner. This time, however, gel was applied to newt foreheads rather than to their nares, prior to testing. If control animals (gel on foreheads) showed strong responses to arginine, but adults with blocked nares did not, then the nasal cavity would be implicated as the principal chemoreceptive site. After each trial, gel was removed and the adult marked to prevent recapture and retesting.

Results

Amino acid composition of worm body fluids

Worm (*Eisenia rosea*) fluids were characterized by high concentrations (262 mmol kg^{-1} body mass) of amino acids (Table 2). Of the 15 compounds assayed, dicarboxylic acids, glutamate and aspartate were the two most abundant, accounting for almost 28% of the total composition. Amino acids with small, non-polar functional groups (alanine and glycine) also were common and made up 20% of the extract. By contrast, lysine and arginine (two basic amino acids) occurred only at lower concentrations. Arginine, in particular, was scarce relative to the other substances. However, a concentration of 5 mmol kg^{-1} mass was roughly 10^6 times higher than in natural stream water at the study site (data not shown).

Description of inactive and chemically-stimulated behavior in the field

The behavior of free-ranging adult newts changed dramatically as a function of stimulus conditions. Without a chemical attractant present, adults lay motionless on the stream bed or swam (or walked) near the bottom, frequently changing direction. There was no tendency for an animal to orient with respect to flow. Once contact was made with a chemical attractant, however, the newt turned and swam rapidly upstream into the plume (Fig. 2). Movement continued along a direct path until arrival at the odor source. Newts often altered the position of their nostrils relative to the bottom. While navigating in and around an odor plume, adults raised their snouts, vertically into the water column, or buried them down into the stream bed. Combined attractant/dye releases showed

Table 2. *Concentrations and percentage compositions of the 15 most abundant free amino acids (L-isomers) in worm body fluids*

Amino acid	Concentration (mmol kg^{-1} body mass)	% composition*
Glutamate	44.5±13.5	17.0±0.1
Aspartate	27.8±10.5	10.6±0.8
Alanine	27.0±7.7	10.3±0.3
Glycine	24.8±7.5	9.5±0.1
Serine	20.1±7.1	7.7±0.4
Leucine	19.7±5.0	7.5±0.4
Valine	17.2±5.2	6.6±0.1
Lysine	16.5±1.8	6.3±1.4
Threonine	14.5±4.9	5.5±0.2
Isoleucine	13.1±4.0	5.0±0.1
Proline	11.7±3.5	4.6±0.1
Phenylalanine	8.4±2.8	3.2±0.1
Tyrosine	6.7±2.4	2.6±0.2
Arginine	5.0±2.4	1.9±0.3
Methionine	4.7±2.1	1.7±0.2
Total	261.7±114.4	100.0

Values are means ± s.e.m.
*Percentage molar concentrations.

that these adults were adjusting the position of their snouts as a means of contacting individual odor filaments within the plumes.

Behavioral responses to arginine and other amino acids

The different amino acids elicited varying responses in newt foraging activity (Fig. 3). A positive behavioral response was here defined as an adult turning upstream and swimming towards the source of chemical release. Of the 13 amino acids tested, three were significantly more active than the stream water control and comparable to worm fluids. There was, however, no significant correlation between amino acid concentration in worm body fluids and percentage of animals responding (Kendall's Tau: τ=0.07, d.f.=10, P=0.79). Furthermore, no significant association was found between percentage of animals responding and either amino acid bulk, hydrophilicity, or charge [see descriptors in Hellberg et al. (Hellberg et al., 1987); Kendall's Tau: $\tau \leqslant 0.18$, d.f.=10, $P \geqslant 0.44$, all three comparisons]. The basic amino acid, arginine, was the most stimulatory compound tested at 10^{-5} mol l^{-1} (Fisher exact test: P=0.003). Further analysis showed that adult newts responded to arginine in a dose-dependent manner (Fig. 4; F-test: $F_{1,3}$=19.9, P<0.0001). The median effective dose (ED_{50}), or threshold, was estimated as 8.3×10^{-7} mol l^{-1} (uncorrected for dilution associated with chemical release and delivery). Alanine and glycine, both short-chain neutral amino acids, also were significantly more attractive at 10^{-5} mol l^{-1} than freshwater alone (P=0.011 and P=0.033, respectively). Taurine, one of three sulfur-containing amino acids tested, elicited elevated levels of adult behavior, although not significantly (P=0.08). This compound differs from other amino acids as it contains an S terminus rather than a C terminus and has a substituted oxygen in place of the amino group. Cysteine and methionine, the other sulfur-containing test solutions, caused 30 and 25% response, respectively – less activity than caused by taurine. Although glutamate did not exhibit significantly higher responses than freshwater controls (P=0.16), it was twice as active as aspartate, a slightly smaller (and more highly charged) amino acid (P=0.40).

Behavioral responses to structural analogs of arginine

Activation of adult foraging behavior was intolerant to even subtle changes in arginine structure. Removal (agmatine), or esterification (*N*-omega-nitro-L-arginine methyl ester), of the

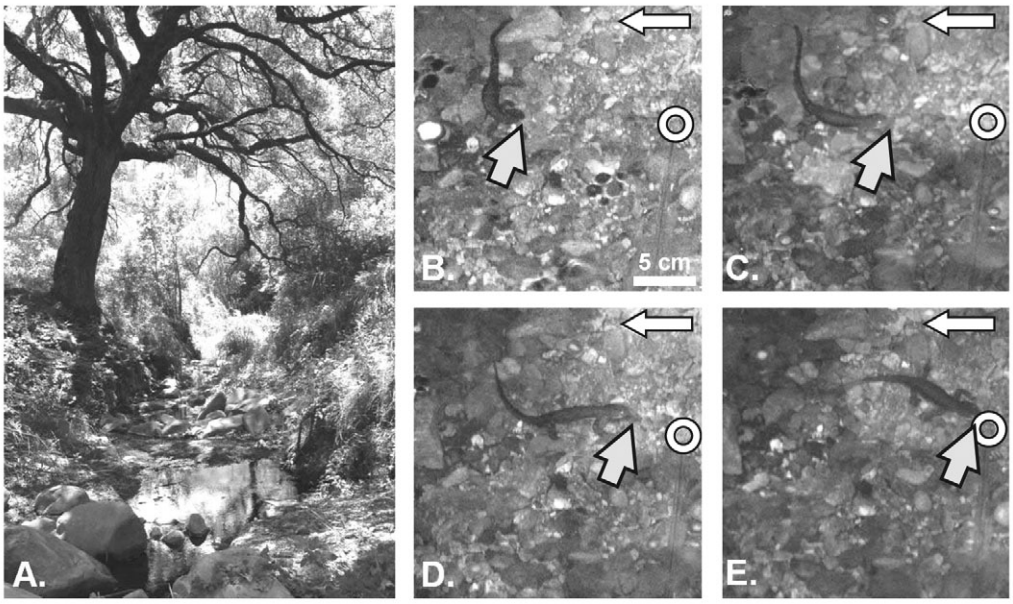

Fig. 2. (A) Tested adult newts inhabited stream pools along a 4 km stretch of Tuna Canyon Creek in Malibu, California, USA. In each 5-min trial, an investigator hid behind rock boulders that lined the stream, and continuously released solution (treatment or control) though transparent polyethylene tubing. The tip of the tubing was placed, nominally, 30 cm upstream of a free-ranging adult newt. (B–E) Successive images (at 3 s intervals) showing an adult (gray arrow) detecting an attractant (arginine, at 10^{-5} mol l^{-1}) odor plume, turning, and swimming upstream to the source (white open circle). The principal along-channel axis of stream flow is denoted by a white arrow.

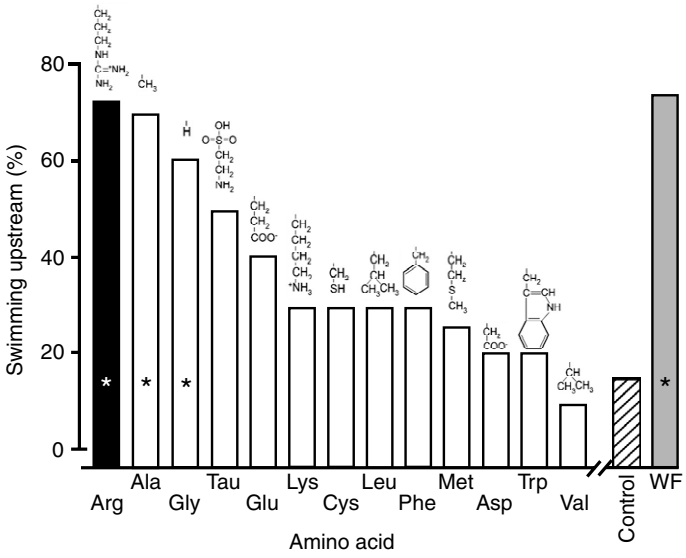

Fig. 3. Percentage of newts swimming upstream to the site of amino acid or stream water (control) release. Each compound was tested at 10^{-5} mol l^{-1}; functional groups are illustrated above respective bars. Arg, arginine; Ala, alanine; Gly, glycine; Tau, taurine; Glu, glutamate; Lys, lysine; Cys, cysteine; Leu, leucine; Phe, phenylalanine; Met, methionine; Asp, aspartate; Trp, tryptophan; Val, valine; WF, worm fluids with a corresponding arginine concentration of 10^{-5} mol l^{-1}). Asterisks denote a significant difference between larval responses to test and control (stream water) solutions, using a Fisher exact test (P<0.05).

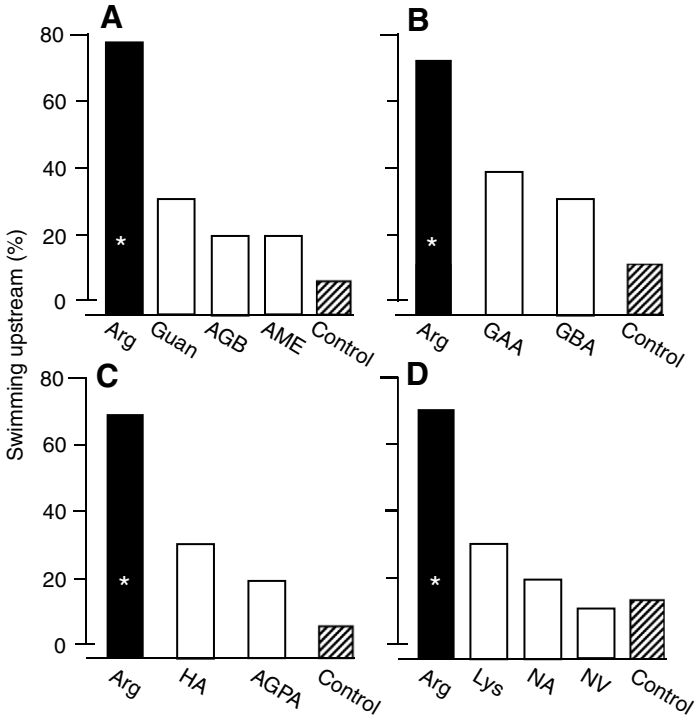

Fig. 5. Percentage of newts swimming upstream to the site of arginine analog or control (stream water) solution release. Each compound was tested at 10^{-5} mol l^{-1}, with separate experiments performed for (A) carboxyl substitutions, (B) amine substitutions, (C) carbon chain substitutions, and (D) guanidinium substitutions. Arg, arginine; Guan, guanidine; AGB, agmatine; AME, arginine methyl ester; GAA, guanidinacetate; GBA, guanidinobutyrate; HA, homoarginine; AGPA, α-amino-β-guanidinopropionate; Lys, lysine; NA, *N*-ω-nitro-L-arginine; NV, norvaline. Asterisks denotes a significant difference between larval responses to test and control (stream water) solutions, using a Fisher exact test (P<0.05).

carboxyl group at the C terminus caused more than a three-fold reduction in the percentage of responding animals (Fig. 5A). Deamination (guanidinobutyrate) also evoked a substantial decrease in behavior (Fig. 5B). Lengthening (homoarginine), or shortening (L-α-amino-β-guanidino-propionate), the arginine side chain by one carbon length reduced activity by over 40% and 50%, respectively (Fig. 5C). Likewise, modification (*N*-ω-nitro-L-arginine), or removal (norvaline), of guanidinium caused a profound reduction in behavioral responses. However, guanidinium, by itself (guanidine), was ineffective in inducing activity (Fig. 5A,D).

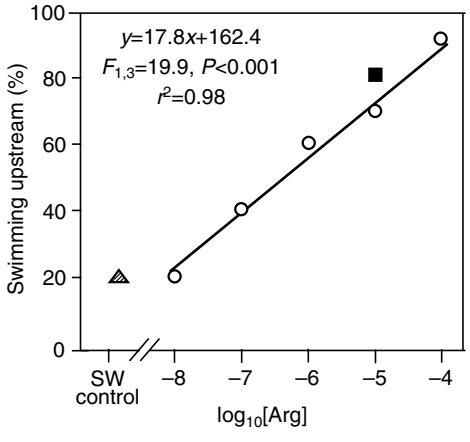

Fig. 4. Effects of concentration of arginine on percentage of newts swimming upstream (open circles) to the site of solution release. Stream water (striped triangle) and worm fluids (closed square; with a corresponding arginine concentration of 10^{-5} mol l^{-1}) served as controls. Concentrations are not corrected for dilution associated with chemical release and delivery.

The role of nasal chemoreception in mediating behavioral responses to arginine

Adult newts possess a nasal cavity, extending from the outer skin surface to the inner roof of the mouth. In behavioral experiments, adults with external nares blocked by inert silicon gel did not react to 10^{-5} mol l^{-1} arginine, whereas control animals immediately swam upstream in response to the same stimulus (Fig. 6; Fisher exact test: P<0.001). Application of stream water had no effect on animals of either group. In adults, the nasal cavity is an important conduit of stimulus-laden water that enables tracking of arginine-scented odor plumes.

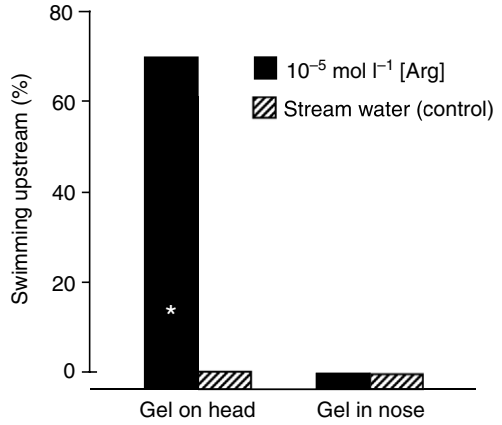

Fig. 6. Percentage of newts swimming upstream to the site of 10^{-5} mol l^{-1} arginine or stream water (control) release. Inert silicon gel was applied either to the forehead (control) or to the nares of newts to block the nasal cavities. Asterisk denotes a significant difference between newt responses to arginine and control (stream water) solutions, using a Fisher exact test (P<0.01).

Discussion

The ontogenetic basis for shifts in chemosensory reception and behavior

The dissolved free-amino acid (DFAA), arginine, has opposing behavioral effects and contrasting ecological consequences on two life history stages of the same newt species (Ferrer and Zimmer, 2007) (current study). It stimulates (adult) predatory search in one case and inhibits (larval) cannibal-avoidance in the other. When exposed to an arginine plume in freshwater streams, adult newts increase locomotion with respect to flow direction (rheotaxis) and odor input source. By contrast, arginine suppresses the stimulatory effects of tetrodotoxin (TTX) on larval swimming, thereby eliminating their cannibal-avoidance reactions (Fig. 7).

Nasal chemoreception explains these contrasting newt behaviors. The occlusion of external nares eliminates adult attraction and larval swimming in response to arginine and TTX, respectively. Both olfactory and vomeronasal organs are housed in the nasal cavity (Døving et al., 1993; Døving and Trotier, 1998). Although vomeronasal sensory cells of some reptiles detect arginine (Hatanaka, 1990), chemosensitivity to this compound in amphibians is attributed at present to olfaction (Vogler and Schild, 1999; Manzini and Schild, 2004).

Reorganization of the olfactory epithelium at metamorphosis thus may explain opposing behaviors to the same compound. As a consequence of life-history stage, profound changes occur in salamander olfactory epithelial morphology (Getchell et al., 1984; Stuelpnagel and Reiss, 2005) and electrophysiological responses to applied chemical stimuli (Arzt et al., 1986). Moreover, behavioral tests on California newt larvae and adults in response to arginine analogs suggest distinct chemosensory receptor populations in each life-history stage. For a common suite of analogs, the magnitudes of adult attraction and larval suppression were not positively correlated (Kendall's Tau: τ=–0.25, d.f.=5, P=0.57). In fact, suppression of TTX-induced behaviors in larvae was tolerant of most structural modifications (Ferrer and Zimmer, 2007). Changes in the carboxyl terminus and carbon side chain had no effect on response to arginine. The only compound that failed to dampen larval responses significantly was lysine, indicating that the guanidinium group is vital for receptor interactions. By contrast, adult behavior was strongly influenced by even subtle alterations in the parent molecule. Minor changes to arginine, such as esterification of the carboxyl group or addition (or deletion) of a single carbon atom to the side chain, destroyed all bioactivity in adults.

Similar transformations in chemoreception are observed in frogs and toads. As a function of larval age, olfactory receptor neurons in *Xenopus laevis* increase specificity for certain amino acids. This finding suggests an ontogenetic shift in patterns of receptor protein expression (Manzini and Schild, 2004). Furthermore, frog olfactory epithelium and olfactory bulb undergo extreme reorganization to accommodate the transition from a totally aquatic larva to a semi-terrestrial adult form (Altner, 1962; Freitag et al., 1995; Reiss and Burd, 1997). Unlike larvae, adults must detect hydrophilic waterborne stimuli, as well as hydrophobic airborne substances. At metamorphosis, nasal cavity morphology is refined and physiological properties are modified, resulting in two distinct classes of olfactory receptor neurons (Freitag et al., 1995; Reiss and Burd, 1997). Whereas Class I receptors are specialized for detecting water-soluble compounds, Class II receptors detect only gaseous volatiles in adults. Ultimately, new central projections connect Class I receptor cells to the ventrolateral olfactory bulb in adults (Reiss and Burd, 1997; Gaudin and Gascuel, 2005).

The role of arginine in mediating trophic interactions

The chemosensory basis for animal perception of DFAAs, including arginine, has been studied extensively for more than 50 years (Steiner, 1957; Case, 1964; Johnson and Ache, 1978; Caprio and Byrd, 1984; Ellingsen and Døving, 1986; Derby et al., 1996; Sorensen and Caprio, 1998; Speca et al., 1999; Valentinčič et al., 2000; Luu et al., 2004). These compounds are renowned feeding stimulants and attractants for diverse aquatic and terrestrial predatory species (for reviews, see Carr, 1988; Bernays and Wcislo, 1994; Carr et al., 1996; Zimmer and Butman, 2000; Markison, 2001; Rogers and Newland, 2003). With the exception of arthropods and teleost fish, DFAAs are, however, taken up rather than released by live, intact, marine and freshwater animals (Manahan et al., 1983; Stephens, 1988; Wright and Manahan, 1989; Manahan, 1990; Zimmer et al., 1999). Species in 18 classes, representing 13 phyla, remove DFAAs from sea-, stream- or lake-water (e.g. Pearse and Pearse, 1973; Crowe et al., 1982; Manahan et al., 1982; Rice and Stephens, 1987; Qafaiti and Stephens, 1988; Lesser and Walker, 1992; Baines et al., 2005). Even in cases where total DFAAs and arginine are released from live, intact, aquatic arthropods and teleost fish, rates are extremely low and, therefore, unlikely to attract predators within native environments (Zimmer et al., 1999).

By contrast, total DFAA and arginine concentrations are

especially high within the tissues and blood/hemolymph of marine and freshwater invertebrates (1–500 mmol kg^{-1} body mass) (Edwards, 1982; Dooley et al., 2002; Hiong, 2004), including common prey (insects, worms and snails) of adult newts (Stebbins, 1972; Hanson et al., 1994). Once compromised by injury or abrasion, invertebrate prey tissues leach total DFAAs at fluxes of 0.07 to 7 mmol min^{-1} kg^{-1} (body mass). At these rates, the prey act as natural attractants to flesh-eating scavengers and predators within aquatic habitats (Zimmer et al., 1999). Single cuts (with a knife blade) that penetrated worm (*Eisenia rosea*) integument caused total DFAA release at 1–4 mmol min^{-1} kg^{-1} (body mass), and, more specifically, arginine discharge at 0.02–0.07 mmol min^{-1} kg^{-1} (body mass) (R.K.Z., unpublished data). This flux of arginine, when scaled proportionally for actual worm body mass (1 g per individual), was almost identical to that introduced by the syringe pump assembly in the present field experiments (with release at 10^{-5} mol l^{-1} and 10 ml min^{-1}). Thus, following release from physically abraded or injured worms, arginine potentially functions as a natural stream-borne attractant to predatory adult newts.

Mechanisms of injury are often associated with processes that affect prey densities. Landslides, for instance, are common during winter and spring months along the banks of southern California mountain streams. They bring large subsidies of terrestrial/fossorial invertebrates, especially worms (*Eisenia rosea*), into nearby freshwater habitats (Kerby and Kats, 1998) (R.P.F., unpublished observations). These events dramatically increase local stream invertebrate populations, and facilitate injury and death to displaced terrestrial animals. Adult newts feed on such subsidies in response to chemical cues, while ignoring conspecific larvae (Kerby and Kats, 1998). This switching behavior of adults may significantly reduce cannibalism, promote coexistence between individuals of different life-history stages, and therefore stabilize newt populations. Arginine release from tissues of compromised worm prey may partly or entirely trigger the switching from larval to alternative prey. Total DFAA and arginine concentrations in amphibian tissues and blood are 10–20 times, or more, lower than those in worms, insects, and other stream invertebrates (Gallardo et al., 1994; Emelyanova et al., 2004). Consequently, these molecules are much more likely to signal the presence of injured invertebrates than conspecifics.

Arginine may well determine predator-prey interactions at multiple trophic levels. Streams of the southern California coastal mountains are numerically dominated by insects (Cooper et al., 1986; Dudley et al., 1986) (R.P.F., unpublished data). Moreover, densities of insect predators are often positively associated with their invertebrate prey (Hildrew and Townsend, 1977; Walde and Davies, 1984; Williams et al., 1993; Kratz, 1996) (but see Peckarsky and Dodson, 1980; Feltmate et al., 1986; Peckarsky, 1991). Given the dose-dependent response of adult newts to arginine, they would be attracted to rich patches of invertebrate prey that summarily sustain high densities of attacking predators, including carnivorous insects. This trophic cascade could, in turn, have significant effects on community structure. Adult newts foraging on invertebrate prey may directly contribute to species-specific population declines. Conversely, newt consumption of insect predators may increase survivorship of invertebrate prey that are predators and prey for other species. In this manner, potentially complex trophic interactions within natural food webs are instigated by chemicals – like arginine – released from compromised prey.

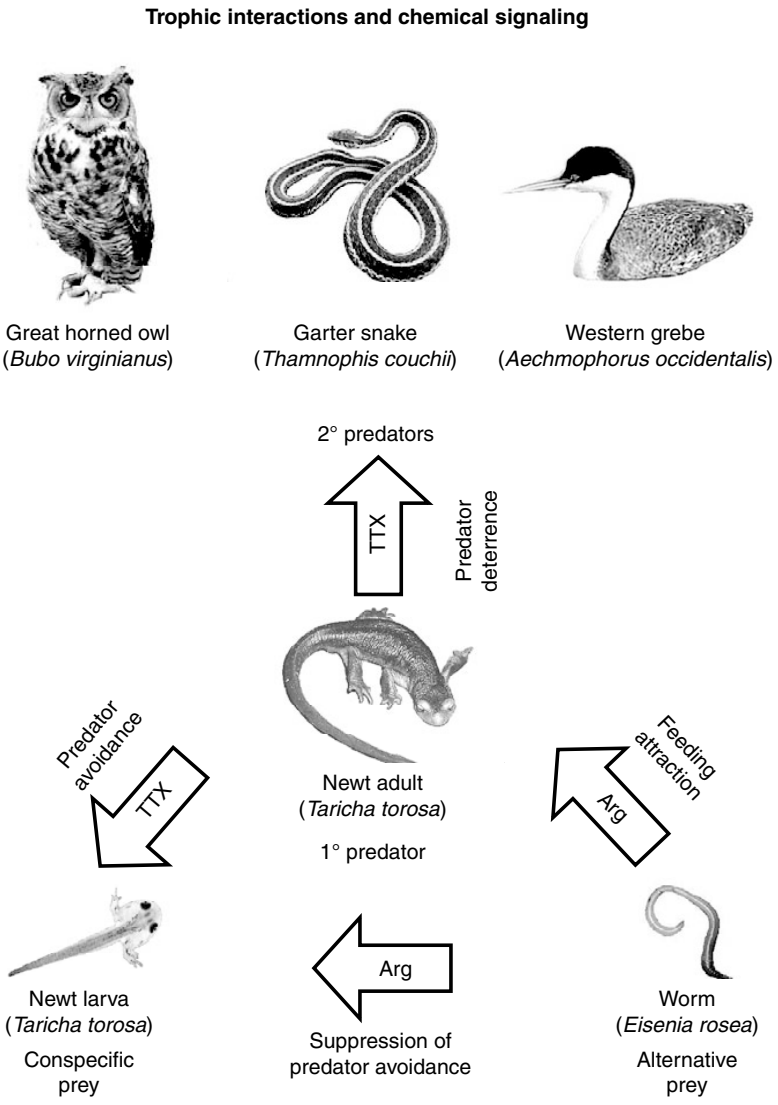

Fig. 7. Schematic depiction of predator–prey interactions involving *Taricha torosa*, and the chemosensory cues that mediate them. Arg, arginine; TTX, tetrodotoxin. This graphic summarizes combined results from eight published investigations (Elliot et al., 1993; McAllister et al., 1997; Kerby and Kats, 1998; Mobley and Stidham, 2000; Brodie et al., 2005; Zimmer et al., 2006; Ferrer and Zimmer, 2007) (present study).

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