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Relating olfactory neurotoxicity to altered olfactory-mediated behaviors in rainbow trout exposed to three currently-used pesticides☆

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Abstract

Odor-evoked neurophysiological responses can form the basis for behavioral responses. Here we first characterized olfactory-mediated behavioral and neurophysiological responses of juvenile rainbow trout to the amino acid L-histidine, then looked at whether there were similar responses to the carbamate antisapstain IPBC and the herbicides atrazine and Roundup®, and lastly explored how exposures to these pesticides modified the L-histidine responses. Trout were behaviorally attracted to 10^{-7} M L-histidine (as assayed in a counter-current olfactometer), but this preference behavior switched to indifference with higher histidine concentrations. Neurophysiologically, the summed electrical responses of peripheral olfactory neurons, as measured using electro-olfactogram (EOG), was 0.843 ± 0.252 mV to 10^{-7} M L-histidine. Of the pesticides, only Roundup® evoked EOGs, indicating the amino acid-based pesticide may have acted as an odorant, and generated a behavioral response: it was avoided at active ingredient [AI; glyphosate isopropyl amine] concentrations $\geq$ 10 mg/l. With 30 min pesticide exposures, 10^{-7} M L-histidine preference behavior was eliminated following exposure to 1 μg/l IPBC and atrazine, and 100 μg/l AI Roundup®. Similarly, 10^{-7} M L-histidine-evoked EOGs were significantly reduced by exposure to 1 μg/l IPBC, 10 μg/l atrazine, and 100 μg/l AI Roundup®. When combined together, the results demonstrate that typical preference behavior can be abolished when neurophysiological responses are reduced by >60% of control. This asymmetry in response thresholds suggests that behavioral responses may be more sensitive toxicological endpoints than neurophysiological responses.

Keywords: Rainbow trout; Behavior; Electro-olfactogram; Amino acid; Atrazine; Roundup

1. Introduction

The loss of olfactory sensory neuron (OSN) response has been used as an ecologically relevant sublethal toxicological endpoint for salmonids (Winberg et al., 1992; Baldwin et al., 2003; Jarrard et al., 2004; Sandahl et al., 2004; Baldwin and Scholz, 2005). The rationale being that since olfaction enables many indispensable behaviors throughout the salmonid lifecycle, including kin and conspecific recognition (Griffiths and Armstrong, 2000; Olsén et al., 2002), alarm response (Mirza and Chivers, 2003), feeding (Hara, 2005, 2006), imprinting and homing (Hasler and Scholz, 1983; Dittman and Quinn, 1996), gamete release synchronization (Moore and Waring, 1996a), and contaminant avoidance (Folmar, 1976; Hansen et al., 1999a,b), its impairment represents a survival threat. Given the ecological, cultural and commercial significance of salmon, and the endangered status of some stocks (COSEWIC, 2002; NMFS, 1996), understanding how contaminants alter this critical sense is of importance. Unfortunately, few studies have related changes in olfactory neurophysiological response to altered olfactory-mediated behaviors.

Several studies on a variety of teleost species have demonstrated the significance of olfaction to behaviors by outright removal of OSN input. For example, severing the OSN-brain connection eliminated the contaminant avoidance of medaka (*Oryzias latipes*) (Hidaka and Tatsukawa, 1989) and reduced the alarm response in matrinxã (*Brycon cephalus*) (Ide et al., 2003). Copper exposure caused loss of OSN tissue (verified through scanning electron microscopy) and eliminated the alarm reaction of Colorado pikeminnow (*Ptychocheilus lucius*) (Beyers and Farmer, 2001). In tilapia (*Oreochromis mossambicus*), cau-

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terization of male OSNs eliminated the behavioral response to pre-ovulatory females (Miranda et al., 2005). Olfactory occlusion has been shown to reduce the migratory fidelity of both chinook salmon (*Oncorhynchus tshawytscha*) (Groves et al., 1968) and black rockfish (*Sebastes inermis*) (Mitamura et al., 2005). In contrast to the above, common surface-water contaminants are known to diminish but not eliminate OSN responses, and these too can have behavioral consequences. For example, the organophosphorus insecticide diazinon has been shown to reduce Atlantic salmon (*Salmo salar*) OSN responses (Moore and Waring, 1996b) and impair two olfactory-based behaviors (anti-predator response and migratory fidelity) in chinook salmon (Scholz et al., 2000).

In the present study we took a top-down (i.e. higher to lower order) approach to explore the interaction between olfactory-based behavioral and neurophysiological responses in pesticide-exposed rainbow trout (*O. mykiss*), a ubiquitous salmonid. We assayed behavioral followed by neurophysiological responses to an amino acid, a natural odorant routinely used in fish olfaction work (see the review by Baldwin and Scholz, 2005). We then tested whether trout avoided three current-use pesticides, and whether the pesticides evoked a neurophysiological response. Finally, we used the three pesticides to help characterize the relationship between neurophysiological and behavioral impairment. Two of the pesticides that we evaluated are among the most widely used herbicides in the world: atrazine and Roundup®. Atrazine is a selective triazine used to control broadleaf and grassy weeds. Although it is considered a next generation 'soft' pesticide (i.e. few non-target species effects and short half-life), it is a known contaminant of aquatic ecosystems (Frank et al., 1990; Pennington et al., 2001). Roundup® is a broad-spectrum, non-selective, post-emergent herbicide also used in the control of broadleaf weeds. The active ingredient of Roundup®, glyphosate isopropylamine salt, is not considered to persist or contaminate aquatic ecosystems (Giesy et al., 2000). However, we tested Roundup® because of its broad use in areas adjacent to salmon habitat in British Columbia (Verrin et al., 2004). Furthermore, the active ingredient of Roundup® is sometimes used directly in aquatic environments to control invasive weeds (Kilbride and Paveglio, 2001). The third pesticide evaluated (IPBC) is a carbamate fungicide used to control mildew (sapstain) on dimensional (i.e. milled) lumber. It is considered moderately persistent (Juergensen et al., 2000) and is highly toxic to aquatic life (Farrell et al., 1998). All three pesticides have been identified as affecting olfactory neurons in salmonids (atrazine: Moore and Lower, 2001; glyphosate: Tierney et al., 2006; IPBC: Jarrard et al., 2004), yet the manner in which the neurotoxic effects relate to behavioral change remains unknown.

2. Methods

2.1. Animals

Rainbow trout (mass 32.7 ± 1.2 g, fork length 14.7 ± 0.18 cm, condition factor 1.01 ± 0.01; $n = 216$) were purchased from Sun Valley Trout Farm (Mission, BC), and maintained in indoor 170 l tanks supplied with filtered, dechlorinated municipal water (background water; dissolved O_2 at >90% saturation, pH 6.8, hardness 6.12 mg/l $CaCO_3$). Fish were kept under a 12-h light:12-h dark photoperiod and fed commercial salmon pellets (EWOS, Surrey, BC) ad libitum. Room and water temperature were synchronized at ~12 °C. All protocols were approved by Simon Fraser University's Animal Care Committee.

2.2. Chemicals

All chemicals were purchased from Sigma–Aldrich (Oakville, Ont.), except for Roundup®, which was purchased from a local retail outlet. The chemicals and their given purities where available are atrazine (2-chloro-4-(propylamino)-6-ethylamino-*s*-triazine; 97.4%), IPBC (3-iodo-2-propynyl-*N*-butylcarbamate; 97%), L-histidine ($C_6H_9N_3O_2$; ≥98.5%), Roundup® concentrate (143 g/l active ingredient [AI] *N*-(phosphonomethyl) glycine isopropylamine salt; Monsanto Company, Canada, Lot #130313/5), and 2-phenoxyethanol.

2.3. Behavioral and electrophysiology apparatus

2.3.1. Preference/avoidance trough

This apparatus is similar to that used by Saglio et al. (2001). In brief, the trough measured 30 cm (width) × 29.5 cm (height) × 154 cm (length) (136 l volume), and was constructed of 1.27 cm thick clear Plexiglas. To provide a discontinuous symmetrical polar gradient, 10 l header tanks were situated at either end, providing a flow of 22.3 l/min, which exited through a perforated center stand pipe. Trough sides were covered by 6 ml black poly. To monitor fish position, five CNL-100 CCD surveillance cameras were spaced above and evenly along the length of the trough. To prevent outside interference, the trough and cameras were enclosed in 6 ml black polyethylene plastic. The cameras were connected to PV-140V surveillance cards in a 2 gigahertz Pentium PC running Surveillance Solutions software (Matco, St. Laurent, Que.).

2.3.2. Neurophysiology measurements

Electro-olfactograms (EOGs), which measure changes in nasal epithelial voltage that are due to a summed generator potential response of OSNs, were recorded after Evans and Hara (1985) using the apparatus described in Jarrard et al. (2004). In brief, fish were anesthetized using 2-phenoxyethanol (0.5 ml/l induction, 0.25 ml/l maintenance) placed in a holder, and the left olfactory rosette was surgically exposed. After surgery, the rosette was kept continuously irrigated with background water or a pesticide/vehicle mixture at a flow rate of ~1.5 ml/min. EOG was measured as the relative change in voltage between two Ag/Ag–Cl electrodes (silver wire suspended in 2% agar and 1 M NaCl inside a borosilicate capillary tube), one situated in the rosette and one on top of the head. The rosette electrode position was established by advancing the electrode until it depressed the mediocaudal area of the rosette raphe and then backed off until no depression was observed. In an effort to limit interpreparation variation, EOG electrode tips were not pulled and so all had a tip diameter 580 μm. EOGs were elicited