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Contaminant-specific targeting of olfactory sensory neuron classes: Connecting neuron class impairment with behavioural deficits

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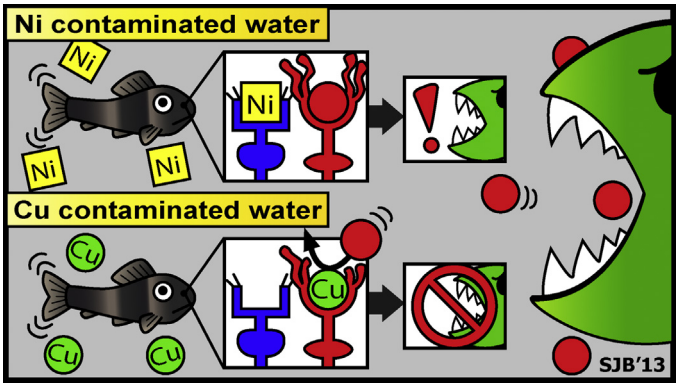
HIGHLIGHTS

- Copper and nickel impair the olfactory system of fathead minnows and yellow perch.
- Copper and nickel have a different effects on ciliated and microvillous OSNs.
- Copper, but not nickel, impairs antipredator response in fathead minnows.
- Response to an antipredator cue in fathead minnows depend on ciliated OSNs.

GRAPHICAL ABSTRACT



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ABSTRACT

The olfactory system of fish comprises several classes of olfactory sensory neurons (OSNs). The odourants L-alanine and taurocholic acid (TCA) specifically activate microvillous or ciliated OSNs, respectively, in fish. We recorded electro-olfactograms (EOG) in fathead minnows (*Pimephales promelas*; a laboratory-reared model species) and wild yellow perch (*Perca flavescens*) whose olfactory chambers were perfused with either L-alanine or TCA to determine if OSN classes were differentially vulnerable to contaminants, in this case copper or nickel. Results were consistent in both species and demonstrated that nickel targeted and impaired microvillous OSN function, while copper targeted and impaired ciliated OSN function. This result suggests that contaminant-specific effects observed in model laboratory species extrapolate to wild fish populations. Moreover, fathead minnows exposed to copper failed to perceive a conspecific alarm cue in a choice maze, whereas those exposed to nickel could respond to the same conspecific cue. These results demonstrate that fathead minnows perceive conspecific, damage-released alarm cue by ciliated, but not microvillous, OSNs. Fish living in copper-contaminated environments may be more vulnerable to predation than those in clean lakes owing to targeted effects on ciliated OSNs.

1. Introduction

The olfactory epithelium of fish comprises a variety of cell types, including olfactory sensory neurons (OSNs) (Zielinski and Hara, 2006). Water bathes the olfactory epithelium and odourants in the water can bind to and activate OSNs, which then transmit

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information about the external environment to the brain where a behavioural response can be mounted (Hamdani and Døving, 2007). There are three types of OSNs found in the olfactory epithelium of fish; crypt, ciliated, and microvillous (Zielinski and Hara, 2006), which are distinguished by morphological differences and, in some cases, by their response to various odours (Hansen et al., 2003). The response of specific classes of OSNs to different odours can be determined by exploiting a difference in the olfactory signalling pathways in different OSN types, namely that crypt and ciliated OSNs have a cAMP-mediated olfactory signalling pathway while microvillous OSNs have an IP3-based pathway (Hansen et al., 2003; Michel et al., 2003; Rolen et al., 2003). This difference in the olfactory signalling pathways among OSNs was exploited to demonstrate that in the round goby (*Neogobius melanostomus*), taurocholic acid (TCA), a bile salt, induces a response specific to OSNs with a cAMP-mediated pathway (i.e., ciliated and/or crypt OSNs), while L-alanine induces a response specific to OSNs with an IP3-mediated pathway (i.e., microvillous OSNs) (Laframboise and Zielinski, 2011). Bile salts from different fish species do not activate crypt cells, and the specificity of TCA to ciliated cells has been demonstrated via histology (Vielma et al., 2008; Døving et al., 2011; Bazáes and Schmachtenberg, 2012). Work in goldfish has demonstrated that when microvillous cells are intact, fish respond to amino acids but not to TCA (Kolmakov et al., 2009). These results support the conclusion that the olfactory response to L-alanine is mediated by microvillous OSNs, while TCA induces a response specifically in ciliated OSNs. The fact that certain odours induce a response specific to an OSN class means that the status of either ciliated or microvillous OSNs can be measured to determine how each OSN class is affected by contaminants.

Odours can induce a variety of behavioural responses in fish, for example fathead minnows (*Pimephales promelas*) avoid conspecific, damaged-released alarm cue, first described by von Frisch (1938). Chemicals released from fathead minnows whose skin has been ruptured will induce stereotypical antipredator behaviour in other fathead minnows (Smith, 1992; Kats and Dill, 1998). The perception of alarm cues, as well as food, mating, and migratory cues, confer an adaptive benefit to the receiver and is vital to survival. Thus, any impairment of this ability to detect and avoid predators may prevent a prey fish from avoiding predation (Carreau-Green et al., 2008).

A variety of contaminants (e.g., metals and pesticides) are known to impair the olfactory acuity of fish (Scott and Sloman, 2004; Pyle and Mirza, 2007; Tierney et al., 2010). The effect of copper on the olfactory system of fish has been studied at the neurophysiological and, to a lesser extent, the behavioural level. At the neurophysiological level, copper has been shown to reduce olfactory acuity in fathead minnows with exposures ranging from 10 min to 96 h (Green et al., 2010; Dew et al., 2012). Coho salmon (*Oncorhynchus kisutch*) also show decreased olfactory acuity when exposed to low concentrations of copper (Baldwin et al., 2003; Sandahl et al., 2007; McIntyre et al., 2008). The behavioural effects of copper mirror those seen at the neurophysiological level as low concentrations of copper inhibit the antipredator response of coho salmon (Sandahl et al., 2007; McIntyre et al., 2012) and Colorado pikeminnows (*Ptychocheilus lucius*) (Beyers and Farmer, 2001). Copper, therefore, can impair the olfactory system at multiple levels of biological organization. The effect of nickel on olfaction, on the other hand, has been poorly studied even though it is commonly found at elevated concentrations in water bodies near areas receiving anthropogenic input (Pyle and Couture, 2012).

Previously we determined that very low concentrations of copper inhibit olfactory acuity in fathead minnows (Dew et al., 2012). However, it was unclear whether copper had a general effect on all OSN classes, or if each OSN class was differentially affected. Histological work with goldfish demonstrated that when exposed to a high concentration of copper sulphate (16 mg L^{-1}) for a short exposure (10 min), ciliated cells were damaged to a greater extent than microvillous cells (Kolmakov et al., 2009). Corresponding neurophysiological measurements showed that when ciliated cells were damaged and microvillous cells were not, response to two amino acids (L-serine and L-arginine) was intact, and after ciliated cells recovered there was again an intact response to TCA (Kolmakov et al., 2009). It is unknown, however, if OSN-specific effects due to copper exposure would occur at more environmentally-relevant concentrations and exposure durations. To determine if copper specifically affects one or more OSN classes, we first confirmed that L-alanine activates microvillous OSNs and TCA activates ciliated OSNs in fathead minnows by measuring the olfactory response using an electro-olfactogram (EOG), a neurophysiological technique that measures the response of the olfactory epithelium of a fish to an odour. We then exposed fish to increasing concentrations of copper and measured their OSN-specific EOG response to TCA (ciliated OSNs) or L-alanine (microvillous OSNs). Nickel was used as a second contaminant to determine if the effect of copper was a generalized effect of metal exposure, or specific to copper. The effect of copper and nickel on EOG response in wild yellow perch (*Perca flavescens*) was also tested, using native lake water to make up the exposure water. Comparison of laboratory-reared fish with wild fish in their native water demonstrates whether or not any OSN-specific effects of copper and nickel occur in wild fish populations.

The response of fathead minnows to an antipredator cue was measured under copper and nickel exposure for direct comparison to the EOG measurements. By measuring both the effect of copper and nickel on EOG and the behavioural response to an antipredator cue, a direct connection can be made between a behaviour and OSN classes.

2. Materials and methods

2.1. Animals

Adult (1.8–4.1 g) fathead minnows were obtained from the USEPA (Duluth, MN) and housed in static renewal or re-circulatory systems in the Lakehead University Biology Aquatic Facility. Fish were held in dechlorinated Thunder Bay, Ontario municipal water (SI-Table 1) with a 16 h photoperiod. Fish were fed ad libitum once daily with *Artemia* spp., and were allowed to acclimate for a minimum of 2 weeks prior to being used in experiments. Alkalinity was determined as previously described (Pyle et al., 2005). All water samples were collected post-exposure in tubes that were rinsed a minimum of three times with the water to be sampled. For metal analysis, samples were acidified using concentrated trace metals grade nitric acid (Fisher Scientific, Toronto, ON, Canada) and filtered through a 0.45 μm filter. Metal concentrations and dissolved organic carbon were measured by ALS Environmental (Thunder Bay, ON, Canada), a laboratory accredited by the Canadian Association for Laboratory Accreditation (CALA). Metal concentrations were measured using inductively coupled plasma mass spectrometry in accordance to all CALA QA/QC guidelines.

Yellow perch (5.2–8.0 g) were collected from Geneva lake in the Sudbury ON region by angling and acclimated to laboratory conditions for 24 h in Geneva lake water (SI-Table 1). Water collection and analysis was as previously described (Azizishirazi et al., 2013).

2.2. Electro-olfactogram experiments

Electro-olfactogram experiments were performed as previously described (Green et al., 2010). The EOG responses to 10^{-3} M L-alanine (MP Biomedicals, Solon, OH, USA) and 10^{-4} M TCA (Fisher