

AVOIDANCE RESPONSE OF A TERRESTRIAL SALAMANDER (*Ambystoma macrodactylum*) TO CHEMICAL ALARM CUES

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Abstract—Organisms from a wide variety of taxonomic groups possess chemical alarm cues that are important in mediating predator avoidance. However, little is known about the presence of such alarm cues in most amphibians, and in particular terrestrial salamanders. In this study we tested whether adult long-toed salamanders (*Ambystoma macrodactylum*) showed an avoidance response to stimuli from injured conspecifics. Avoidance of stimuli from injured conspecifics could represent avoidance of a chemical alarm cue or, alternatively, avoidance of a territorial pheromone or conspecific predator odor. Consequently, we also tested whether salamanders avoided stimuli from noninjured conspecifics. Salamanders avoided stimuli from injured but not from noninjured conspecifics. Therefore, we concluded that the response to injured conspecifics represents avoidance of a chemical alarm cue and not avoidance of a territorial pheromone or predator cue. This is the first clear demonstration of chemical alarm signaling by a terrestrial amphibian and the first report of chemical alarm signaling in an ambystomatid salamander. By avoiding an area containing stimuli from injured conspecifics, long-toed salamanders may lower their risk of predation by avoiding areas where predators are foraging.

Key Words—Alarm pheromone, chemical signaling, predator avoidance, long-toed salamanders, *Ambystoma macrodactylum*.

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INTRODUCTION

A wide variety of taxa have been shown to detect and respond to chemical alarm signals (alarm pheromones) released by conspecifics. Such signals can be released by the sender either before or after a predator captures the prey (e.g., Smith, 1992; Wisenden et al., 1995a,b). Signals that are released upon mechanical damage to the sender (known as damage-release alarm signals) should act as reliable indicators of predation risk in that they indicate that a conspecific in the immediate vicinity was recently attacked or captured by a predator. By avoiding areas where predators have recently captured a conspecific, prey should be able to lower their probability of being captured.

Damage-release chemical alarm signals have been documented in various taxa (insects: Sih, 1986; Chivers et al., 1996; gastropods: Snyder, 1967; Appleton and Palmer, 1988; echinoderms: Parker and Schulman, 1986; Lawrence, 1991; sea anemones: Howe and Sheikh, 1975; crayfish; Hazlett, 1994; fishes: Smith, 1992; amphibians: see below). Except for fish, there is relatively little information on the distribution of alarm pheromones among the vertebrates. For example, few studies have been conducted on amphibian alarm pheromones. Eible-Eibesfeldt (1949), Hrbacek (1950), and Kulzer (1954) described an alarm response to stimuli from injured conspecifics in common toad (*Bufo bufo*) tadpoles. In laboratory experiments, tadpoles exposed to conspecific stimuli typically swam to the bottom or to the side of the tank opposite from where the pheromone was introduced. In field experiments, tadpoles avoided alarm pheromones by swimming into deeper water. Pfeiffer (1966) tested nine species of larval anurans in five different families. He found responses in two species of bufonid tadpoles (*Bufo bufo* and *B. calamita*), and speculated that alarm pheromones may be restricted to the family Bufonidae. Alarm pheromones are also known from another bufonid, the western toad (*Bufo boreas*) (Hews and Blaustein, 1985; Hews, 1988). Recent studies have shown that alarm pheromones may also be found in larval ranid frogs, including Cascades frog tadpoles (*Rana cascadae*) (Hews and Blaustein, 1985, but see Hokit and Blaustein, 1996) and red-legged frog tadpoles (*Rana aurora*) (Wilson and Lefcort, 1993).

Only two studies have investigated the possibility of chemical alarm signals in salamanders. Lutterschmidt et al. (1994) showed that adult plethodontids, *Desmognathus ochrophaeus*, avoid skin extracts from conspecifics and congeners, *D. brinleyorum*. This may represent an avoidance response to chemical alarm cues. However, this response may also represent avoidance of territorial pheromones or predator odors. Other plethodontid salamanders have been shown to avoid territorial pheromones and odors of predatory plethodontids (e.g., Jaeger, 1986; Roudebush and Taylor, 1987). In another experiment that tested for the presence of alarm signals in salamanders, Marvin and Hutchison (1995)

demonstrated that two species of adult semiaquatic newts (fire-bellied newts, *Cynops pyrrhogaster* and red-spotted newts, *Notophthalmus viridescens*) avoided the area of a test chamber containing moistened paper towel that was saturated with skin extracts from conspecifics. Since adult newts are not territorial and are not preyed upon by other newts or salamanders, Marvin and Hutchison (1995) concluded that the avoidance response represented avoidance of a chemical alarm cue.

In this study we investigated the avoidance response of the terrestrial form of the long-toed salamander (*Ambystoma macrodactylum*) to stimuli from injured conspecifics. Although adult long-toed salamanders are not known to be territorial or to prey on conspecifics, we also tested the salamanders for an avoidance response to chemical stimuli from noninjured conspecifics. An avoidance response to stimuli from noninjured conspecifics may indicate avoidance of territorial pheromones or conspecific predator odors and would complicate our interpretation of an avoidance response to stimuli from injured conspecifics. However, if adult long-toed salamanders are shown to avoid chemical cues from injured conspecifics but not from noninjured conspecifics, our study would be the first to clearly show chemical alarm signaling by a terrestrial amphibian.

METHODS AND MATERIALS

Collection and Maintenance. In November 1995, we collected adult long-toed salamanders on or beside a 200-m section of Oakville road, located approximately 8 km east of Corvallis, Benton County, Oregon. Each salamander was maintained individually in a Petri dish (150 mm diameter \times 25 mm deep) that contained a moistened paper towel. The salamanders were maintained on a 14L:10D photoperiod at approximately 16°C and were periodically fed with crickets. The salamanders were not fed for three days prior to testing.

Experimental Protocol. We tested the avoidance response of individual salamanders to three different stimuli: (1) chemical stimuli from injured conspecifics, (2) chemical stimuli from noninjured conspecifics, and (3) a combination of chemical stimuli from both injured and noninjured conspecifics. Testing the response of salamanders to a combination of stimuli from injured + noninjured conspecifics was done in order to allow us to speculate on the strength of an avoidance response to either injured or noninjured stimuli. To prepare the injured conspecific stimulus we killed six animals by decapitation and removed their viscera, spinal column, and legs. The remaining tissue (approximately 12.0 g, which contained mostly skin, but also some muscle tissue) was ground with a mortar and pestle, combined with 200 ml of dechlorinated tap water and filtered through a fine mesh net. We used 5 ml of injured conspecific stimulus per trial.

This solution was used within 15 min of preparation. For a source of chemical stimuli from noninjured conspecifics, we used moistened paper towels that had housed a single conspecific for 48 hr.

For each trial we lined half of a rectangular plastic container (32 × 18 × 8 cm) with a paper towel that was moistened with dechlorinated tap water (control side). The treatment side of the containers was lined with paper towel that contained stimuli from injured conspecifics, stimuli from noninjured conspecifics, or a combination of both. The two paper towel substrates in each container were separated by approximately 2 cm to reduce diffusion of chemicals between the sides. After the appropriate stimuli were added to each side of the test containers, we used a spray bottle containing dechlorinated tap water to saturate the paper towels. This ensured that any observed avoidance of the control or stimulus sides could not be attributed to differences in moisture level between the sides.

At the start of each trial, we introduced a single test salamander into the center of the container. Every 3 min for 2 hr we recorded whether the test salamander was located on the control or treatment side of the container. If the salamanders were positioned across the middle of the containers, we used the position of the salamanders' snout to assign location. To control for the possibility of a bias in the salamanders' orientation in the room, we rotated the containers 180° every half hour during the experiment.

Twenty different salamanders were tested in each of the three treatments for a total of 60 tests in this experiment. Individual salamanders were never used in more than one test. We size-matched the salamanders for tests in the three treatments. The snout-vent length of salamanders was 45.0 ± 7.9 mm (mean \pm SD). For each trial we summed the number of times each salamander was located on the treatment side of the container out of a possible 40 observations (one observation every 3 min for 120 min = 40 observations). For each of the three treatments, we used a binomial test (Siegel and Castellan, 1988) to compare the number of trials in which the salamanders spent less than 50% of their time on the stimulus side.

RESULTS

In trials using stimuli from noninjured conspecifics, there was no difference in the number of trials in which test salamanders chose the control side over the stimulus side of the testing chamber (Table 1, Figure 1). However, in both the injured conspecific and injured + noninjured conspecific treatments, the test salamanders showed a significant avoidance of the treatment side of the containers (Table 1, Figure 1).

TABLE 1. NUMBER OF TRIALS IN WHICH TEST SALAMANDERS CHOSE STIMULUS AND CONTROL SIDES OF EXPERIMENTAL CONTAINERS^a

Treatment	Trials (<i>N</i>) where test animals spent majority of time		<i>P</i>
	Stimulus side	Control side	
Noninjured	12	8	0.504
Noninjured + injured	5	15	0.042
Injured	3	17	0.002

^aSee text for details, *P* values are based on two-tailed binomial tests.

DISCUSSION

The results of our study show that adult long-toed salamanders avoid chemical stimuli from injured conspecifics. Given that salamanders did not avoid stimuli from noninjured conspecifics, the avoidance response to injured conspecific stimuli cannot be interpreted as an avoidance response to territorial pheromones or predator odors. As a result, we conclude that the avoidance response of the salamanders to injured conspecifics represents avoidance of a chemical alarm cue. This is the first clear demonstration of chemical alarm signaling in a terrestrial amphibian and the first report of chemical alarm signaling in an ambystomatid salamander.

We found that salamanders exposed to stimuli from injured conspecifics spent more time on the control (dechlorinated water) side of the testing chamber than salamanders exposed to stimuli from injured + noninjured conspecifics. The intermediate response of salamanders to the combined stimuli from injured + noninjured conspecifics, in relation to either the noninjured or injured treatments alone, indicates that the salamanders may have a conflicting response to the combined stimuli. In the combined treatment, the presence of noninjured stimuli appeared to have weakened the intensity of the avoidance response to the injured conspecific stimuli. Nevertheless, salamanders still exhibited a significant avoidance of alarm cues in the combined stimulus treatment.

We do not know the identity of the chemical(s) that act as the alarm cue in long-toed salamanders. Nevertheless, the cue is likely contained in the skin (and/or possibly muscle tissue) as these were the tissues we used to prepare the injured conspecific stimulus. Kulzer (1954) suggested that bufotoxin, a predator deterrent contained in the skin of bufonid tadpoles, may serve as the alarm cue for bufonid tadpoles. In his study, bufotoxin (bufodienolides) and γ -bufotoxin

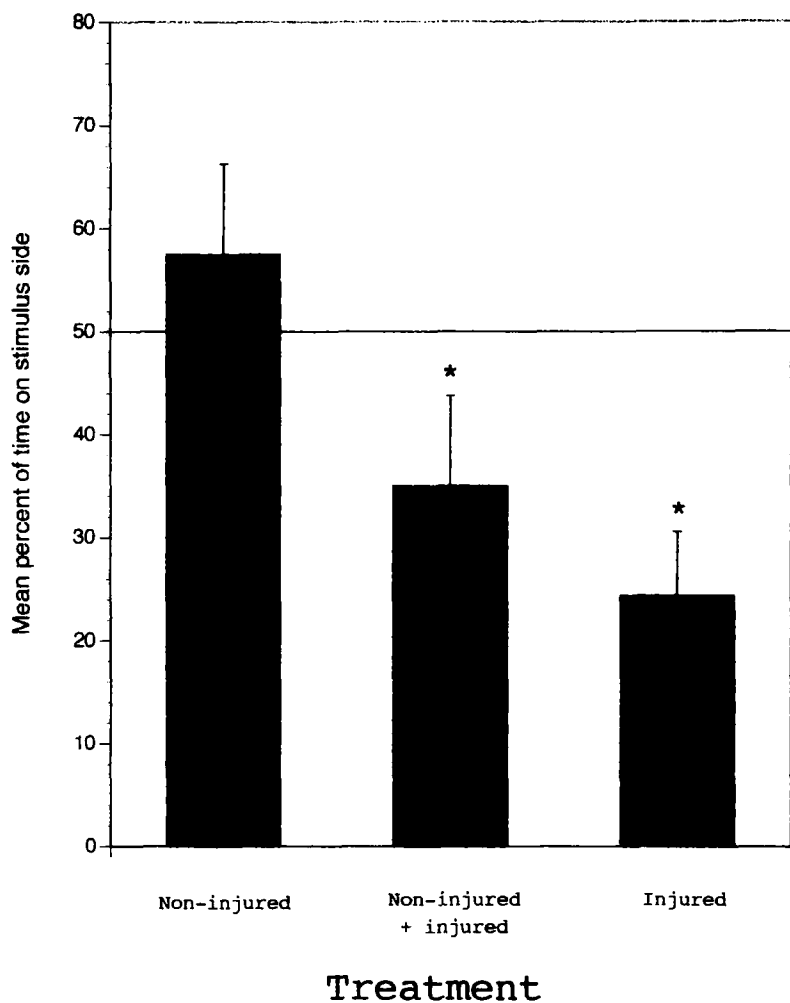


FIG. 1. Mean (+ SE) percentage of time test salamanders spent on the stimulus side of the experimental containers. Means and SE are used to illustrate trends in the data only. The data were analyzed with two-tailed binomial tests. *Significant difference at $P < 0.05$.

were as effective as chemical stimuli from injured tadpoles in eliciting alarm responses in toad tadpoles. Lutterschmidt et al. (1994) suggested that pseudotritontoxin (PTTX), a toxin contained in plethodontid salamanders, may function as the alarm cue in *Desmognathus ochrophaeus*. Ambystomatid salamanders are

known to possess toxins in their skin (e.g., Brodie and Gibson, 1969; Mason et al., 1982). As in other amphibians, these chemicals may also serve a dual function as both predator deterrents and alarm cues.

The behavioral response we quantified was avoidance of the alarm cue. This is a common response of a variety of prey to the presence of alarm cues (e.g., Lutterschmidt et al., 1994; Chivers and Smith, 1994). By avoiding an area where a predator has recently captured a conspecific, the prey should be able to lower their risk of predation by avoiding the predator's foraging microhabitat. Under natural conditions, multiple exposures of prey to alarm cues in specific microhabitats may facilitate recognition of the area as dangerous. It is unknown whether such an effect occurs in amphibians. However, some prey fishes are able to learn the identity of dangerous microhabitats based on the presence of alarm cues (Chivers and Smith, 1995; Wisenden et al., 1995a).

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