## Tests of Motion Vision and Phototaxis in Salamander Larvae

Carl W. Schneider, Barbara W. Marquette and Paul Pietsch

Department of Psychology
Indiana University of Pennsylvania
Indiana PA 15705
and
Department of Visual Sciences
School of Optometry
Indiana University
Bloomington, IN 47405

The larval salamander's visual detection of moving patterns and its phototaxic tendencies are difficult to appraise objectively and quantify rigorously. Stone (1930) waved a model worm over the bowl to ascertain whe-

ther or not vision had been reestablished following heteroplastic transplantation of eyes in larvae. Aside from the fact that this approach exposes the animals to patterns of movement unlike those displayed by natural prey, observer bias may contaminate the results.

Monte Carlo Worm Test. In examining motion vision, we have sought to circumvent the aforementioned problems by introducing the larvae into a quasi-natural environment we call the Monte Carlo Worm Test, where the observations are recorded on a time-lapse video cassette (and thus removed from direct experimenter intervention) while the animal has an opportunity to respond to visually salient but inaccessible living prey; viz. Enchytreaus chytra worms, one of the voraciously carnivorous larva's standard food sources.

For worm testing, animals are placed in a 20 cm diameter Stendor dish containing 6 radially arranged, equally spaced liquid scintillation vials, one of which is randomly chosen to contain worms just before the trial; trials

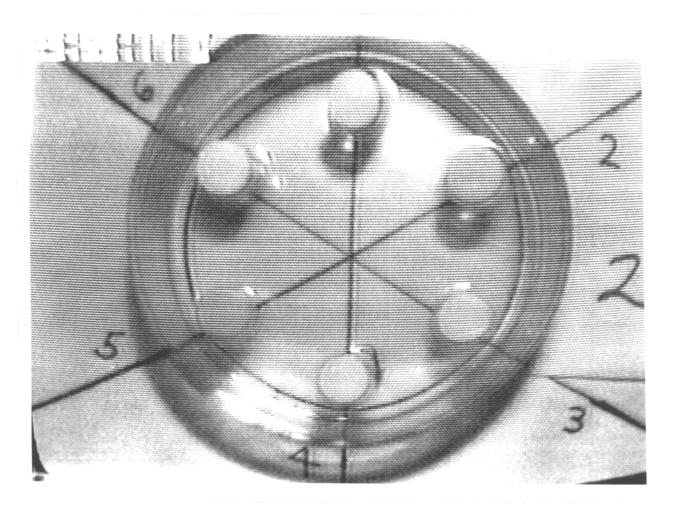


Figure 1. Video freeze-frame of an A. punctatum larva being worm-tested (note vial 4.)

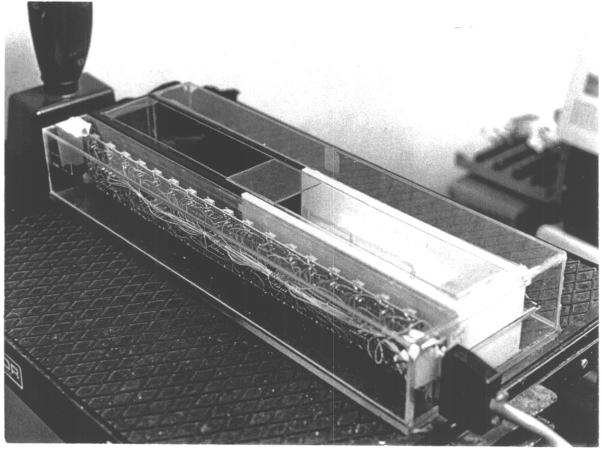


Figure 2. Automated light/dark testing apparatus.

are usually of 2 hours duration. Alerted to the white, wriggling, thread-like worms, a typical larva (e.g., A. punctatum) approaches and repeatedly encircles the target while intermittently executing strike or attack movements, as if attempting to break into the vial. Within 15 minutes most animals habituate to the would-be quarry and cease hovering and attacking, typically spending the remainder of the trial circumnavigating the perimeter of the bowl, without returning to the worm vial.

Figure 1 shows a video freeze-frame of a trial in progress with a subject at vial 4, the worm vial in this instance. We can run the time-lapse tape in real time and score one hour's worth of recorded behavior in 87 seconds. Thus, in addition to a highly reliable yes/no spot check of the subject's motion detecting ability, we can quantify its reaction with parameters such as latency to approach and time spent in the sector of the worm vial. The chances of an animal's randomly choosing the worm vial statistically approach those of repeatedly winning at roulette. (The latter inspired the design and name of the test.)

Animals subjected to anterior decerebration (under MS 222 narcosis) display a dramatically different response (Pietsch and Schneider, 1990). Decerebrated subjects show little tendency to habituate during a 2-hour test period. Their response is a pattern of intense, relentless hovering and attacking. Occasionally, they will briefly leave the worm vial, soon to return and resume the stereotypical hovering and attacking, despite no chance of reaching the reward.

**Phototaxis.** Phototaxic tendencies in larval salamanders have been studied primarily in natural habitats, but little attention has been directed to the phenomenon in the laboratory (Schneider, 1968; Duellman and Trueb, 1986). We have approached phototaxis by employing a computerized infrared monitoring system (Kirkpatrick et al, 1991) designed to record the larva's locomotor patterns. A subject is placed in a rectangular 47.5 cm long by 5.1 cm wide alley (see Fig. 2), half-dark and half-light, containing 17 equally spaced infrared sensors that are scanned every 120 milliseconds for 2 hours. A data acquisition program, written in ASYST, allows us to collect concurrent information on the animal's: a) location anywhere in the alley during any in-

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stant; b) movement patterns; c) activity levels.

So far we have "light-dark" tested four species of Ambystoma larvae, in addition to various fishes. We have found that A. mexicanum larvae (axolotls) of wild type pigmentation show no strong phototaxic tendencies, while A. tigrinum, A. punctatum, A. opacum, and the mutant albino axolotl (a/a) exhibit vigorous and statistically significant negative phototaxis. Enucleating both eyes or severing both optic nerves (all nociceptive procedures are carried out under MS 222 narcosis) imme-

diately eliminates negative phototaxis. However, negative phototaxis recovers within about two weeks following an optectomy; te., after partial regeneration has occurred. The typical response in the monitor is illustrated in Figure 3, which depicts the data of two negatively phototaxic A. punctatum larvae. "Dark" and "Light" in the illustration represent dark or light halves of the alley. Notice how the trend is decidedly towards the left (infrared sensors 1 and 2) and as far from the light as possible.

## Phototaxic Response

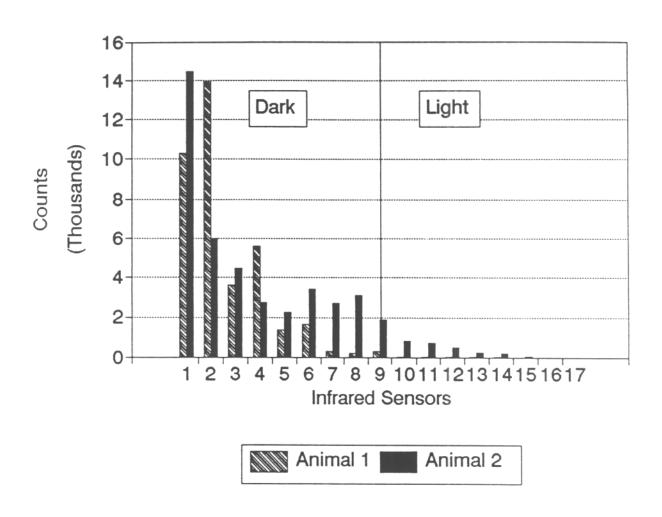


Figure 3. Computer generated bar graph displaying the number of times an animal was detected at a specific infrared sensor. The alley was scanned every 120 ms for two hours (60,000 scans).

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