Landmark learning by the Ozark zigzag salamander

Plethodon angusticlavius

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Abstract Although salamanders have been shown to respond to classical conditioning, spatial learning has been largely unstudied. We tested whether salamanders could learn to locate foraging areas by using landmarks. We trained 10 salamanders Plethodon angusticlavius to use landmarks (small rocks) to locate patches within the arena containing food (blackworms Lumbriculus variegatus). At the corners of each square testing arena were four plastic dishes, one containing blackworms and the other three empty. A rock was placed in front of the dish containing blackworms, and the location of the food-dish was randomly chosen for each training trial. A control group was also trained to feed on blackworms in the presence of a rock, but the rock was positioned randomly among the four dish locations so that the rock was not a reliable landmark for the worms. Although the length of the training period for individual salamanders varied (22–38 trainings per individual), the mean number of trainings for salamanders in the control and experimental groups was equal (30 training trials). During testing, no blackworms were present to eliminate any visual or chemical cues emanating directly from the prey. Individuals trained with the rock landmarks spent significantly more time in the area of the landmark than did control salamanders [Current Zoology 57 (4): 485–490, 2011].

Keywords Landmark learning, Spatial learning, Salamander learning, Plethodon angusticlavius, Beacon

One common mode of learning is by classical conditioning where animals make associations between a familiar stimulus and a novel/neutral stimulus. For instance, some animals can learn information about predators by associating the scent of an unfamiliar predator with that of a known frightening stimulus (fish: Chivers and Smith, 1994; mammals: Griffin et al., 2001). In the same way animals can learn spatial information by associating characteristics of important resources such as shelters or patches of prey with specific features of the environment (“landmarks”). Use of familiar landmarks for orientation also has been referred to as piloting (Schmidt-Koenig, 1965) or Griffin’s Type I orientation (Griffin, 1952). A wide variety of species have been shown to be capable of some degree of landmark learning, including insects (bees: Cartwright and Collett, 1983; ants: Collett et al., 2001), octopus (Mather, 1991), fish (Odling-Smee et al., 2008), frogs (Adler, 1980), reptiles (lizards: Day et al., 1999, Zuri and Bull, 2000; snakes: Holtzman et al., 1999; turtles: Lopez et al., 2001), birds (Gibson and Kamil, 2001) and small mammals (Collett et al., 1986).

Landmark learning is likely to be particularly important for individuals whose home ranges are fixed and relatively small so that various locations can be easily revisited. In addition, the resource must be either stationary (e.g., burrows or other shelters, water sources) or predictably renewable (e.g., patches of prey) so that the landmark is a reliable indicator of the presence of the resource. The natural history of territorial terrestrial salamanders (genus Plethodon) fits with these criteria. During moist conditions, salamanders forage on the forest floor where food is abundant, but under dry conditions they are restricted to patches of moisture under rocks and logs (Jaeger and Barnard, 1981). For the red-backed salamander Plethodon cinereus, core areas of territories around cover objects are extremely small (less than 0.5 m²: Mathis, 1991), and the wider foraging area is also limited (13–24 m²: Kleeberger and Werner, 1982). Salamanders occupy the same cover objects throughout the active season and sometimes across seasons (Gergits and Jaeger, 1990; Mathis, 1990), so it is likely that being able to learn the location of at least some resources is important. In addition to the location of cover shelters, the location of particularly profitable patches of prey may be important. These salamanders have broad diets composed of virtually any appropriately-sized invertebrate (Petranka, 1998). The location of forest-floor invertebrates is largely influenced by microclimate (Rykken et al., 2007), and so areas with

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appropriate microhabitat characteristics are likely to be reliable locations for particular prey items.

In previous studies amphibians have been found to learn non-spatial information. Hepper and Waldman (1992) showed that frogs learned novel food scents as embryos, and Ferrari et al. (2007) documented social learning by frog larvae. Salamanders (Order: Caudata) have also demonstrated learning. For example, salamanders have been trained through classical conditioning to respond to unfamiliar predators (Crane and Mathis, in press; Woody and Mathis, 1998). Salamanders are also able to learn recognition of novel objects (Hershkowitz and Samuel, 1973), recognition of novel food sources (Gibbons et al., 2005), and avoidance of negative stimuli (Ray, 1970). Additionally, salamanders have learned to respond to high predation risk as embryos (Mathis et al., 2008). Although salamanders have been shown to learn various sources of information, their spatial learning abilities have received very little attention.

The Ozark zigzag salamander Plethodon angusticlavius is a lungless salamander that lives under leaf litter, logs and rocks in the deciduous forests of northern Arkansas and southern Missouri. These salamanders are completely terrestrial; individuals hatch from eggs as miniature adults and establish territories when they are older (Mathis and Britzke, 1999). In this study we hypothesized that Ozark zigzag salamanders could learn to use landmarks to locate foraging patches. We used a testing protocol that was pioneered by Tinbergen (1932), who demonstrated that digger wasps, Philanthus triangulum, can learn to associate the location of their burrows with surrounding landmarks (pinecones). This protocol was referred to as the “transformational approach” by Cheng and Spetch (1998). Because our protocol examines attraction of individuals to a single landmark that is directly associated with the resource, it can be categorized as a test of a mechanism called “beacon homing”, which was described by Gallistel (1990).

1 Materials and Methods

Ozark zigzag salamanders (n = 20; SVL = 38.2 ± 0.62 mm and mass = 0.54 ± 0.03 g) were collected from Bull Shoals Field Station in Taney County, Missouri, USA. These salamanders were housed in the laboratory in large square chambers (plastic Petri dishes; 23 × 23 cm and 2.5 cm height) and were fed live blackworms Lumbriculus variegatus three times per week. Salamander holding chambers were kept inside an environmental chamber at 18 °C with a 12:12 light:dark cycle.

For our protocol to be successful, it was necessary to confine the prey to a “patch”. We used circular plastic dishes (PVC knock-out plugs; 19 mm radius and 9 mm height), each containing 1–2 ml of water and one blackworm, as food patches. The worms did not escape these dishes. Because this method of prey presentation was novel, salamanders were given pre-experimental feeding trials to become accustomed to feeding from the dishes. Once a salamander successfully fed from the dishes, we entered it into training trials. The length of the pre-experimental period varied for each individual (1–4 weeks). We alternated assignment of training groups (10 experimental salamanders and 10 control salamanders) so that both groups were equal in experience consuming blackworms, but individuals began training on different dates.

We trained the salamanders in their home arenas about every two days from 20 May – 7 September 2009 between 10: 30 and 16: 00 h at 23–26 °C under fluorescent overhead lighting. We placed one dish at each corner of the arena with only one dish containing a prey item (one blackworm, 1–3 cm length). The other three dishes held water but no blackworms (Fig. 1A). A small rock (mass range: 1–2 g) was also present in each arena. In the arenas of salamanders in the experimental group, the rock was always adjacent to the food-dish holding the blackworm (i.e. it was a reliable predictor of the presence of food). The rock was positioned about 5 mm from the food-dish and between the dish and the center of the container. The placement of the prey patches and the rock occurred while the salamander was covered by an opaque circular holding container (PVC knock-out plug; 25 mm radius and 9 mm height) that blocked visual cues. Salamanders were kept inside the holding container for 5 min before it was removed and the salamander was free to explore the arena. We randomly selected which of the four dishes would contain the blackworm and adjacent landmark each training trial; the other three dishes had neither blackworms nor adjacent rocks. Because assignment was random, possible use of landmark cues external to the salamander’s chamber was eliminated. A control group was also trained to feed on blackworms in the presence of a rock, but the positions of the blackworms and the rocks were randomized independently (using a randomized block procedure) so that the rock was not a reliable landmark for the worms (Fig. 1B). Thus, for control salamanders, the rock spent roughly equal amounts of time placed clockwise, counter-clockwise, diagonally, and adjacent.
to the dish containing food. Before testing, individual salamanders in both experimental and control groups received an average of 27.5 ± 2.29 training sessions ($t = 0.0$, $P = 1.0$).

All 20 salamanders were tested on each of two testing days: 28 August and 9 September 2009 at 11:30–13:00 h when laboratory temperatures were approximately 23°C. Each salamander remained in its home arena for testing, and a transparent grid was placed over it. The grid had four square quadrants (8 × 8 cm²), and each surrounded one of the dishes. To eliminate any visual or chemical cues from the food, none of the dishes contained blackworms during testing. The salamander was placed under a holding container, and the rock landmark was randomly placed adjacent to one of the empty dishes (Fig. 1C). The salamander was kept in the holding container for 5 min and then was free to leave the central area and enter into one of the quadrants. Testing trials lasted 30 min; we recorded the location of the salamander (landmark quadrant, quadrant without landmark, or no choice) every minute based on the location of the pectoral girdle of the salamander. The response variables we analyzed were the latency to enter the landmark quadrant, the total number of times the landmark quadrant was chosen (out of 30 recordings), and the percentage of times the landmark quadrant was chosen (no. of landmark quadrant choices / no. of total quadrant choices).

We averaged the data from the two testing dates for each individual to avoid pseudoreplication.

2 Results

Our data met the assumptions for parametric statistical testing, so we used two-sample t-tests. Salamanders that had been trained to use landmarks spent significantly more time in the quadrant with the landmark during testing (number of times the landmark quadrant was chosen: $t = 3.37$, $P = 0.005$, Fig. 2A; percentage of times the landmark quadrant was chosen: $t = 3.20$, $P = 0.007$, Fig. 2B). On average the landmark-trained salamanders also moved to the landmark quadrant about

![Fig. 1 Experimental methods for landmark learning by salamanders](image)

A. Training arena for experimental group salamanders: landmark always adjacent to food. B. Training arena for control group salamanders: landmark randomly placed in relation to food. C. Testing arena with landmark present but no food.

![Fig. 2 Summary of results from learning tests](image)

A. Mean (±SE) numbers of times the landmark quadrants were chosen in learning tests by Ozark zigzag salamanders *Plethodon angusticlavius* that were trained to use landmarks (experimental group) and those that were not (control group). Two-sample t-test: $t = 3.37$, $P = 0.005$. B. Mean (±SE) percentages times the landmark quadrants were chosen in learning tests by Ozark zigzag salamanders *Plethodon angusticlavius* that were trained to use landmarks (experimental group) and those that were not (control group). The dotted line represents the random expectation of 25%. Two-sample t-test: $t = 3.20$, $P = 0.007$. 
3 min earlier than control salamanders (11.65 ± 2.21 and 14.60 ± 2.73 min), but these latencies were not significantly different (T = 0.84, P = 0.412).

3 Discussion

Our data support the hypothesis that Ozark zigzag salamanders can learn to use landmarks (i.e. beacon homing) to find foraging patches. Compared to control salamanders, individuals trained with landmarks spent over twice as much time in the quadrant with the landmarks. Salamanders showed substantial variability in their food-searching strategies, with individuals either searching for food immediately, moving quickly to the corner they faced and then slowly beginning to explore, or remaining still for a relatively long period of time and then beginning to explore. Qualitatively, the frequencies of these behavior patterns did not appear to vary between training groups (i.e. there seemed to be bold and shy individuals in both groups). However, these differences in initial individual behavior contributed to the high variation in foraging latencies.

The random expectation was that salamanders would spend an average of 25% of their time in each of the four quadrants. However, salamanders that were trained to associate the food patch with the rock landmark chose the landmark quadrant 37% of the time, which is, as expected, greater than the random expectation (T = 1.94, P = 0.04, one-tailed). Surprisingly, control salamanders appeared to avoid the rock landmark, choosing the landmark quadrant only 13% of the time, which is less than the 25% expected by chance (T = -3.44, P = 0.007, two-tailed). This low frequency of use of the quadrant with the rock landmark could be because control salamanders learned that the rock usually (75% of the time) indicated the site of an empty dish, and, thus, they were more likely to find food in one of the three dishes without an adjacent rock. An alternative explanation is that the control salamanders found the rock to be a generally negative stimulus, but this explanation seems unlikely since salamanders are often found associated with rocks in their natural habitat.

Whether the attraction to landmarks by the experimental salamanders was to a visual or chemical cue or a combination of both is unknown. Although terrestrial plethodontid salamanders tend to be more active at night, they can use visual information for foraging (David and Jaeger, 1981; Placyk and Graves, 2001). Studies on orientation by newts _Salamandra salamandra_ suggest that visual landmarks may be the most important cue for this species (Himstedt and Plasa, 1979; Plasa 1979), but studies of homing in _Plethodon_ have focused on possible use of chemical information (Jaeger et al., 1993; Madison, 1969). The possibility that the salamanders in our study detected airborne cues emanating from the rock landmark is also feasible because volatile cues are known to be important to salamanders in at least some contexts (Dawley, 1984; Martin et al., 2005). Individuals in some species have been found to have the strongest responses when both chemical and visual cues were present (frogs: Stauffer and Semlitsch, 1993; lizards: Amo et al., 2004; crayfish: Bouwma and Hazlett, 2001).

Most studies of associative learning in amphibians have been in the context of predator-recognition learning, and these studies have shown rapid learning of predatory cues, usually with a single training trial (e.g., Crane and Mathis, in press; Ferrari and Chivers, 2008; Mirza et al., 2006; Woody and Mathis, 1998). Rapid learning for avoidance of noxious prey (Rice and Taylor, 1995) and cues associated with harsh environments (heat and drying) (Grubb, 1976) has also been reported. Note, however, that rapid learning does not appear to be the case for all aversive stimuli. Studies that trained amphibians to recognize stimuli associated with either electric shocks or bright lights, stimuli that are aversive but not naturally encountered in their habitats, required numerous training trials (Greding, 1971; Ray, 1970). In contrast to stimuli associated with predatory cues, learning associated with prey has been reported to require a substantial number of training trials (Hershkowitz and Samuel, 1973). We did not collect data during the training period, but our qualitative assessment was that salamanders needed numerous training trials before showing somewhat consistent responses to the rock. One hypothesis to explain these differences is that amphibians may be able to more quickly learn information associated with predation risk than with foraging opportunities, possibly due to the greater fitness consequences to the failure to recognize situations involved with high predation risk in comparison to the consequences of missed foraging opportunities. However, there are at least two alternative hypotheses that should be considered. First, because patches of prey can be either depletive and transient or predictably renewable (Mathis et al., 1995), it may take numerous visits to prey patches for individuals to determine whether associated landmarks are indeed reliable indicators of prey presence. Second, the design of our experiment may have been particularly challenging in comparison to what a salamander might experience in nature because the location of the landmark was not stable; in small
mammals, landmarks that change position are more difficult to learn than stable landmarks (Biegler and Morris, 1993, 1996), and attraction to landmarks can decrease if the position is changed after a period of stability (Devonport and Devenport, 1994).

Terrestrial salamanders can maintain above-ground site-specific territories for several months, with some territories re-occupied by the same individuals even after an over-wintering period when salamanders occupied underground burrows (Gergits and Jaeger, 1990). Therefore, the usefulness of the retention of spatial information, including landmark (beacon) learning, has potential to have important fitness consequences. Individuals that can rapidly locate reliable prey patches on the forest floor are at an advantage to individuals that rely on random search because they can more quickly return to their home shelters under rocks and logs where they are safer from predators and from desiccation.

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