Tiger salamanders' (*Ambystoma tigrinum*) use of features

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ABSTRACT

In their environment, salamanders must avoid both predation and desiccation, necessitating efficient movement throughout their world. Although past research has indicated that salamanders can use visual cues in navigating through their environment, it is not clear how geometric and feature information are incorporated by salamanders in their movement through their surroundings. Past work with a variety of species indicates that geometric information is regularly used and that features are also used under some circumstances. We explored salamanders' use of features when both had been available during initial learning. First, salamanders learned to navigate to a correct corner within a rectangular box with a distinctive column in each corner. Each salamander was assigned a correct corner, and salamanders were trained in acquisition to move toward the correct corner. Following acquisition, salamanders completed two test trials. In one, only geometric cues were available as the features were removed. In the second, the features' placements were jumbled and conflicted with the geometric information. These results suggest that salamanders in this experiment used feature information over geometric information when both had been previously available during acquisition.

1. Introduction

While many studies have examined the cognition of humans, other mammals, and birds, very few researchers have ventured to study the cognitive abilities of amphibians or reptiles (Burghardt, 2013; Shettleworth, 2010; Sotelo et al., 2015). Today, researchers are beginning to recognize that we cannot ignore amphibians and reptiles in our quest to understand cognitive abilities of both humans and nonhuman animals more fully (e.g., Matsubara et al., 2017).

Studies of amphibians and reptiles suggest that their cognitive abilities are likely more sophisticated than first supposed (e.g., Matsubara et al., 2017). For instance, red-backed salamanders (*Plethodon cinereus*) select larger quantities over smaller quantities in a forced-choice discrimination task (Uller et al., 2003) and Oriental fire-bellied toads (*Bombina orientalis*) demonstrate evidence of quantity discriminations for small and large numerosity (Stancher et al., 2015). Studies have also revealed that tiger salamanders (*Ambystoma tigrinum*) and fire salamanders (*Salamandra salamandra*) remember information across brumation, a hibernation-like state (e.g., Kundey et al., 2018; Wilkinson et al., 2017).

Like other amphibians, salamanders must find food as well as avoid both predation and desiccation (Sinsch, 2006). Thus, the ability to move through the world efficiently is essential to survival. Past research suggests that tiger salamanders can learn to use visual cues in navigating through a maze (Kundey et al., 2016). However, it remains unclear how geometric and feature information from the environment might be incorporated in their movement through their surroundings. Such investigations are important to our understanding of cognitive evolution and comparative cognition because amphibians' patterns of development differ substantially from those of birds and mammals.

While this issue has not been systematically investigated in amphibians, it has been addressed in a number of other species. For example, work with chickens (Vallortigara et al., 1990), rats (Cheng, 1986), pigeons (Kelly et al., 1998), fish (*Xenotoca eiseni*; Sovrano et al., 2002), and rain forest ants (*Gigantiops destructor*; Wystrach and Beugnon, 2009; Wystrach et al., 2011), as well as human adults and children (Hermer and Spelke, 1994, 1996), suggests that geometric information is regularly utilized and that featural cues are also used under some circumstances (for review, see Cheng and Newcombe, 2005; Twyman and Newcombe, 2010). Such research has spawned numerous theories and discussion regarding the use and integration of geometric and feature information since Cheng's (1986) seminal work. Thus, the purpose of this study was to explore salamanders' use of feature information when both feature and geometric information were available for use throughout acquisition, although use of feature information was differentially reinforced.

To begin to address this question, we utilized a rectangular box with distinctive columns in each corner (i.e., features). We assigned each

salamander a correct corner (e.g., corner with checkerboard column), and salamanders were trained in acquisition to choose the correct corner, as described below. Cue/corner assignments were consistent for each salamander but distributed randomly across salamanders. During acquisition, salamanders could use geometric information since the box is rectangular, feature information since distinctive cues were placed in each corner, or both feature and geometric information. If the salamanders did not attend to the features (in this case, the columns in the corners), only geometric information would be available; two corners had a long wall to the left and a short wall to the right while the other two corners had a long wall to the right and a short wall to the left. However, while both feature and geometric information were available, salamanders were differentially reinforced for using feature information.

After learning to choose the correct corner during acquisition, we conducted two counterbalanced test trials, as described below. To evaluate use of geometric information, we removed all features for one trial. We hypothesized that if salamanders had attended to geometry, salamanders would choose their originally-assigned corner or its geometric equivalent even when the features were removed. To evaluate use of feature information, we jumbled the features' placements in another test trial. We hypothesized that if salamanders had attended to features, salamanders would follow the column previously associated with their assigned corner even when the features were moved. We compared performance in both test trials to elucidate how salamanders utilized feature and geometric information when both were available during test, although use of features had been differentially reinforced during training. If salamanders were to succeed in both test trials at levels exceeding chance, this would suggest they use both feature and geometric information.

2. Method

2.1. Subjects

The subjects were 22 adult salamanders (Haha Reptiles, Honey Grove, TX); ultimately, 18 subjects completed the experiment. Salamanders were maintained throughout training and testing in their home cages at 23 °C (± 2 °C) and 70% humidity in group aquariums with coconut fibre bedding in a dimly lit laboratory. Outside of rewards received as part of experimental trials, access to freeze-dried insects was provided twice weekly. All procedures accorded with Hood College's Institutional Animal Care and Use regulations.

2.2. Apparatus

A white rectangular box (43.18 cm long × 23.5 cm wide × 46.36 cm high) was used for all training and testing trials (see Fig. 1). Each corner was marked by a distinctive column (5.08 cm long X5.08 cm wide X39.37 cm high) constructed from purple and white duct tape. These included a solid purple column, a purple and white vertical stripe column, a purple and white horizontal stripe column, and a purple and white checkerboard column.

2.3. Procedure

During acquisition, salamanders learned to approach an assigned corner within the box, where each corner was marked by a distinctive column. The assignment of columns to corners was randomly assigned across salamanders but consistent for each salamander.

Prior to each trial attempt, the salamander was placed in a plastic container on a turntable. The salamander was rotated at a rate of 30 rpm for 30 s. Subsequently, each salamander was placed alone in the box's centre. The wall the salamander was facing at the start of each trial was determined randomly. After placing the salamander in the centre of the box, the experimenter moved out of view and watched the
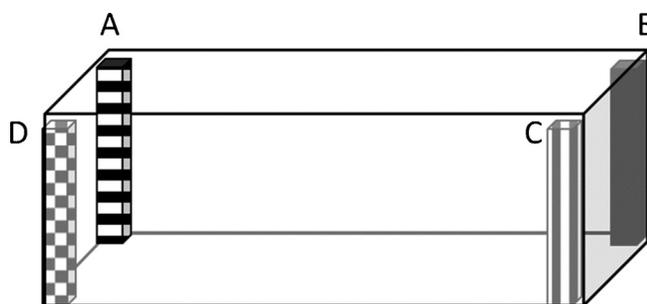

Fig. 1. Depicts training trial apparatus setup. Corners A and C are geometrically identical; corners B and D are geometrically identical. In one test trial, all columns were removed, leaving only geometric information. In another test trial, the columns' placements were systematically jumbled such that the features' placement was inconsistent with the geometric cues available during training and such that no column was in the same corner that it was located in during acquisition or in the geometrically-equivalent corner to where it was placed during acquisition. Test trial order was counterbalanced across animals.

salamander through a baby monitor (Summer Infant 02720). A salamander was counted as approaching a column if its head came within 7.62 cm of the column.

If the salamander moved to the correct corner, the experimenter rewarded it with a portion of a freeze-dried cricket, and it was returned to its home cage. If incorrect, the salamander was rotated as described above before again being placed in the centre of the box to repeat the trial. Salamanders were given up to three attempts each day to make a correct choice. Once a correct choice had been made, the salamander was rewarded with a leg from a freeze-dried cricket in the apparatus before being returned to its home cage. If a salamander took longer than two minutes to respond during a trial attempt, the attempt was counted as incorrect and the salamander repeated the trial as described above.

To move to the test phase, salamanders were required to choose correctly on the first attempt of the day for three consecutive days. After meeting this criterion, we conducted two test trials (order counterbalanced) separated by 24 h. In one test trial, all features were removed; thus, only geometric information remained. In a second test trial, we jumbled the features' placements such that the features' placement during test was inconsistent with the geometrical cues present during training. During tests, care was taken to make sure that no column was in the same corner it was located in during acquisition and that no column was in the geometrically-equivalent corner to where it was placed during acquisition; additionally, this was done systematically to ensure equivalent distribution of possible placements across animals. During test trials, salamanders received a cricket leg for any choice made. However, while salamanders received the cricket leg reinforcement during acquisition immediately after making their choice in the apparatus, we reinforced salamanders during test trials outside of the apparatus in their home cage. Reinforcement was provided in this manner to lessen the likelihood that reinforcement in the first test trial might influence choices in the second test trial. The test trials were otherwise identical to the acquisition trials with the exceptions noted above.

2.4. Statistical analysis

To evaluate salamanders' responses during the test phase, we employed binomial tests where the salamanders were considered to either have made a correct or incorrect choice. For the test in which only geometrical cues were present, we determined the number of salamanders choosing the previously trained corner or its geometrical opposite. Choosing the previously trained corner or its geometrical opposite was counted as correct while choosing either of the other two corners was counted as incorrect. If a salamander chose a corner
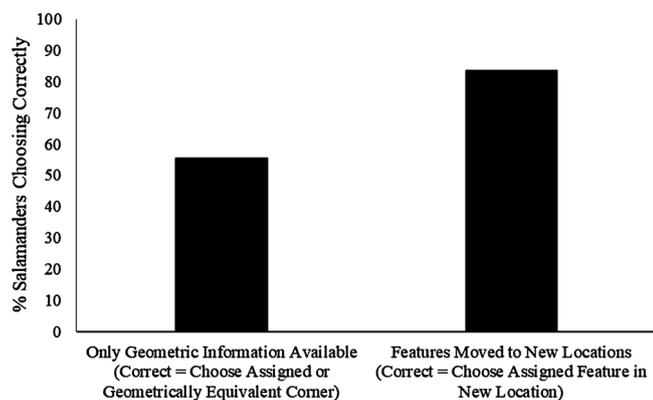
Fig. 2. Depicts performance of salamanders in the two test conditions. The black bars indicate the percentage of salamanders out of 18 salamanders choosing correctly for each of the counterbalanced test conditions. When only geometric information was available, chance performance was equal to 50%. When features were moved to a new location, chance performance was equal to 25%.

randomly for this test trial, it would have succeeded only 50% of the time. For the test in which the features were jumbled, we determined the number of salamanders choosing the correct feature even when its location was moved from that during acquisition; choosing a corner that did not contain the feature previously reinforced was counted as incorrect. If a salamander chose a feature randomly during this test trial, it would have succeeded only 25% of the time since only one feature was correct. Thus, by chance, we might expect only 25% of salamanders to correctly move toward their assigned feature in this test trial by chance alone. Results from the binomial tests were considered significant only if $p < 0.05$.

3. Results

Twenty-two salamanders (10 females, 12 males) started the experiment. Out of the initial 22 animals, 18 salamanders (8 females, 10 males) completed acquisition and both test trials; 4 were eliminated (3 due to death, 1 due to experimental error). Only data from the 18 subjects completing all phases of the experiment were included in statistical analyses. The mean trials to criterion was 61.61 (SD = 49.39, range 16–231); the mean days to criterion was 26.61 (SD = 16.63, range 9–77).

When all features were removed, 10 out of 18 salamanders chose their assigned corner or its geometric equivalent (see Fig. 2). This indicates that salamanders were not statistically more likely than chance to choose their assigned corner nor its geometric equivalent ($p = 0.41$). This suggests salamanders were not readily using the box's geometric information when the feature information was removed during test.

However, 15 of 18 salamanders chose their assigned feature when it was moved to a different location ($p < 0.001$) during test (see Fig. 2). This indicates salamanders learned to follow the feature in navigating to their assigned corner during training.

4. Discussion

These results suggest that salamanders in this experiment used feature information over geometric information when both had been previously available during acquisition, with use of features reinforced during training. Interestingly, in many of the other species tested, geometric information was found to be utilized readily (for review, see Cheng and Newcombe, 2005). For other species, feature information often seems to clarify the geometrical information that subjects use while navigating. Additionally, other species are sometimes unable to use feature information alone under some circumstances. Our results

with salamanders showed that they did not readily use geometry under the experimental conditions we utilized. Instead, they preferentially utilized the feature information they had been reinforced for using during acquisition.

However, our results do not rule out the possibility that salamanders could learn to navigate using geometric information or that they do use geometric information when navigating in their natural environment. First, we reinforced animals for attending to the columns during acquisition. This may have predisposed them to not attend to the geometric information available in the box. Additionally, it is possible that feature information overshadowed learning about the geometric information available during acquisition in this experiment as the features we used were very large. Therefore, it is possible that the columns were so salient to the salamanders that they led to the overshadowing of the available geometric information present within the rectangular box. Thus, if only geometric information was available during acquisition, use of geometric information was reinforced, or the size of the features were reduced during training, it is possible that salamanders would have learned to use the geometric information. It is also possible that removing the columns during the geometry test was a great enough change to the apparatus from acquisition that it disrupted performance; including a test condition with plain columns in a future experiment could help us address this possibility. Use of geometric information might also be made more likely if geometry was the only cue available for success. It is also possible that usage of geometric information might be observed in a more ethologically-valid paradigm. Future experiments might explore each of these issues as well as explore whether similar results are found with other amphibians.

4.1. Conclusion

These results suggest that salamanders in this experiment used feature information over geometric information when both had been previously available during acquisition, although only usage of feature information was reinforced during acquisition. However, these results do not exclude the possibility that salamanders regularly use geometric information in other contexts for navigation or that they could learn to use such information to navigate when other cue types are unreliable or their usage is not reinforced.

Author note

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