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Learning to learn: advanced behavioural flexibility in a poison frog

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Behavioural flexibility is essential for survival in a world with changing contingencies and its evolution is linked to complex physical and social environments. Serial reversal learning, in which reward contingencies change frequently, is a key indicator of behavioural flexibility. While many vertebrates are capable of serial reversal learning, only birds and mammals have previously been shown to use rule-based decision strategies (e.g. win-stay/lose-shift) to become better at learning changes in reward contingencies across reversals. While the lifestyles of many amphibians have a degree of complexity, the evidence to date suggests limited levels of behavioural flexibility. Here, we show that the poison frog *Dendrobates auratus*, which has evolved complex parental behaviours that likely depend on remembering locations in a flexible manner, can use a win-stay/lose-shift strategy to increase their behavioural flexibility across sequential changes in the reward contingencies in a visual discrimination task. Furthermore, probe trials demonstrate that the frogs used the provided visual cues to spatially orient in the maze in a manner reminiscent of complex spatial cognition. Our study provides the first evidence of serial reversal learning in frogs and is the first to demonstrate the use of a rule-based learning strategy in a nonavian, nonmammalian species.

Behavioural flexibility is the ability to change one's behaviour according to variation in the environment, and it can enable animals to increase survivorship and reproductive success (Fagen, 1982; Snell-Rood, 2013). For example, species with greater levels of behavioural flexibility are more likely to successfully invade a new environment (Tebbich, Sterelny, & Teschke, 2010; Wright, Eberhard, Hobson, Avery, & Russello, 2010). Serial reversal learning, in which animals progressively improve their performance in a task with frequently changing reward contingencies, is a standard laboratory method for measuring behavioural flexibility (Bitterman, 1965; Roth & Dicke, 2005), and the ability to perform serial reversals is more often found in animals that live in complex physical and social environments (Bond, Kamil, & Balda, 2007; Godfrey-Smith, 2002; de Waal & Tyack, 2003).

There are several mechanisms that allow animals to learn serial reversal tasks (Gonzalez, Behrend, & Bitterman, 1967; Mackintosh, 1974; Parker et al., 2012; Shettleworth, 2009; Strang & Sherry, 2014). Among them, lower-order processes, such as proactive interference (Bitterman, 1965; Mackintosh, 1974), involve involuntary learning and hence are thought to represent a lower level of behavioural flexibility (Parker et al., 2012; Shettleworth, 2009). These mechanisms have been discovered across a broad range of vertebrate taxa (Gaalema, 2011; Gonzalez et al., 1967; Mackintosh, McGonigle, & Holgate, 1968). In contrast, rule-based strategies, which indicate the ability of an animal to learn and use the underlying rule of the reversal task, represent a greater degree of behavioural flexibility (Parker et al., 2012; Shettleworth, 2009). For example, using a win-stay/lose-shift rule requires animals to make their current choice based on the reward from their previous choice (Mackintosh et al., 1968; Shettleworth, 2009). The optimal outcome of this strategy is the one-trial reversal in which animals make an error on the first trial of a reversal followed by all correct choices on subsequent trials of that reversal (Mackintosh et al., 1968). This type of rule-based strategy has only previously been found in mammals and birds (Mackintosh et al., 1968; Rayburn-Reeves, Stagner, Kirk, & Zentall, 2013; Rumbaugh, Savage-Rumbaugh, & Washburn, 1996; Shettleworth, 2009).

Amphibians, which include both aquatic and terrestrial stages in their life cycle, have to handle environments with a high degree of complexity. Yet, they were once thought to lack behavioural flexibility (Bitterman, 1965; Bitterman, 1975), and have been considered inflexible in learning tasks in artificial laboratory environments

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(Maier & Schneirla, 1935). More recent studies, however, show that amphibians can solve mazes using local visual cues (i.e. visual cues that are directly associated with a goal or are part of the goal), body-centred motor strategies (remembering a place by learning to turn left or right) and geometric cues (i.e. the shape of the space) (Crane & Mathis, 2011; Daneri, Casanave, & Muzio, 2011; Ellins, Cramer, & Martin, 1982; Heuring & Mathis, 2014; Sotelo, Bingman, & Muzio, 2015). Furthermore, in simple discriminations, amphibians are capable of single reversals (Daneri et al., 2011; Ellins et al., 1982; Schmajuk, Segura, & Reboreda, 1980). Nevertheless, we still know relatively little about the cognitive strategies used by amphibians in reversal tasks and whether they are capable of the types of behavioural flexibility observed in mammals and birds.

While many frogs have relatively simple social behaviours, the poison frogs (Dendrobatidae) have evolved complex social and spatial behaviours reminiscent of many mammals and birds (Summers, 1989; Summers & Tumulty, 2013): they are territorial, show mate guarding and pair bonding (some are even monogamous; Brown, Morales, & Summers, 2010), and the parents of some species transport tadpoles to deposition sites (small pockets of water) in the forest canopy after hatching. Some species show homing abilities in the field that suggest advanced spatial cognition (Pasukonis, Warrington, Ringler, & Hödl, 2014). However, whether poison frogs can use spatial cues in a flexible manner and whether they use cognitive strategies similar to birds and mammals is unknown.

We trained the poison frog *Dendrobates auratus* in a two-arm maze in which the position of the correct arm was associated with visual cues in the starting chamber. The visual cues could be reliably associated with the goal based on spatial relationships, but they could not be used for direct guidance (e.g. an animal could not simply approach the visual cues to locate the goal). Our study was designed to (1) determine whether poison frogs could use visual cues to learn a complex spatial discrimination task, (2) investigate whether poison frogs are capable of serial reversal learning and (3) identify the behavioural mechanisms underlying improvement during serial reversal.

METHODS

Animals

We used 10 sexually mature *D. auratus* (four males, six females) that were bred in captivity and were likely several generations removed from the wild (Indoor Ecosystems, LLC, Whitehouse, OH, U.S.A.). In this species, males maintain territories and provide parental care (egg attendance, tadpole transport); females maintain territories and perform mate guarding but do not provide parental care (Summers, 1989). We maintained the animals under conditions that approximated their natural habitat: 25 °C, 80% relative humidity (RH), 12:12 h light:dark cycle (lights on at 0700 hours). We housed the frogs individually in terraria and fed them fortified fruit flies three times per week. The University of North Carolina's Institution for Animal Use and Care Committee approved all procedures (protocol 14-026).

Apparatus

The maze consisted of a central starting chamber and two arms (Fig. 1). The maze arms were uniformly white, but the starting chamber had visual cues on each side (Fig. 1). The frogs were required to use the visual cues in the starting chamber to spatially orient to the goal. We blocked the exits at the end of the arms with identical white doors, only one of which could be opened during a given trial. We attached a rope to the reverse side of the correct door and we blocked the other door from behind with a brick that was not visible to the frog in the maze. We used white absorbent paper, which was replaced every day, as the floor of the maze. Thus, any potential olfactory cues on the floor would be disrupted each day and would not be reliably associated with the goal. We covered the maze with Plexiglas and surrounded the maze with a 1.4 m high white curtain in order to isolate extraneous visual cues in the room. We recorded trials using a camera above the arena (1.5 m high). Experimenters, who were blind to the progress of each individual, sat outside the white curtain to record each training trial and open the door on the correct side. We provided five shelters outside the maze in which the frogs could find refuge after exiting the maze (Fig. 1). To motivate the frogs to locate the exit in order to find shelter, we created a bright, hot (37 °C) and dry (10% RH) environment inside the maze. The frogs are accustomed to a moist environment with ample shelter, similar to the forest floor, and, as such, they find the bright, open environment of the maze to be aversive. Therefore, the reward for finding the correct door was to gain access to a shelter and then the home cage.

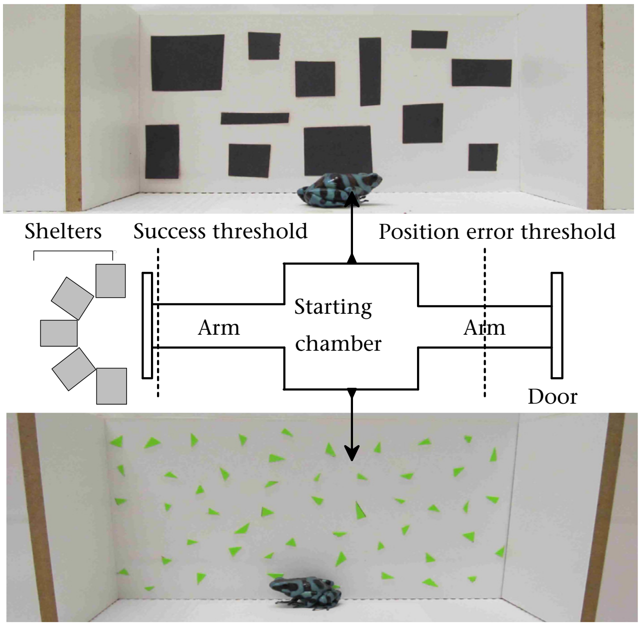

Figure 1. Schematic drawing of the two-arm maze (54 cm (L) × 18 cm (W) × 9.5 cm (H)) and photos of the visual cues on the interior walls of the starting chamber.

Procedure

Acclimation

Before training, we acclimated the frogs to the maze in two trials approximately 24 h apart. During acclimation, both doors were open and no shelters were provided. We used a small, overturned pot with a cardboard floor to transfer and release the frogs in the middle of the starting chamber, resulting in an unpredictable orientation of the frog at the start of each trial. All frogs appeared highly motivated to leave the maze and successfully exited within 2 min.

Acquisition

For the initial learning trials (acquisition), we arbitrarily determined which door was correct. We trained the frogs with three trials per day with an intertrial interval greater than 1 h (from 60 min to 80 min). We wiped the apparatus with alcohol after all individuals had finished one trial. As frogs could be in any position