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Reconsidering sex differences during place learning in túngara frogs

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Abstract

In túngara frogs, female mate choice requires remembering the location and/or calls of preferred males who advertise from fixed positions within a breeding pond. A previous study found that, when solving a place discrimination task in the laboratory, female túngara frogs were able to learn a visual cue to solve the task, whereas males were not. In that task, male performance appeared to be inhibited, in part, by their attempt to use egocentric cues. We tested whether the sex difference in place learning previously reported would generalize to other training parameters with different cues available by eliminating the potential to use egocentric cues and increasing the number of trials per day. As before, frogs were given a choice between a red or yellow door, one of which led to shelters and return to their home cage. In the current testing conditions, we detected a preference for the red door; thus, we only considered frogs rewarded to the yellow door. Training was associated with an increase in correct choices and an increased preference for the yellow door. However, there was no evidence for a sex difference in learning. In summary, under the current training conditions, we found that the apparent female advantage in place learning was no longer evident. Future studies that investigate sex differences in cue preference and/or ability to switch among cues will further illuminate the conditions under which sex differences in learning are manifest in túngara frogs.

Key words: allocentric cue, egocentric cue, local cue, place learning, túngara frog

Sexual selection is one of the most important evolutionary processes for producing sex differences. According to the adaptive specialization hypothesis, sex differences in cognition can be attributed to different demands for solving cognitive problems (Geary 1995; Jonasson 2005; Dalla and Shors 2009). This hypothesis has been confirmed in a number of species. For example, male meadow voles, who keep bigger home ranges than females, committed fewer errors than females in learning a maze task (Gaulin and Fitzgerald 1989). Female cowbirds, who are required to remember locations of hosts' nests, located food rewards with fewer errors and using a more direct path than males who do not have such a requirement (Guigueno et al. 2014). Female túngara frogs, who must remember the location and/or calls of preferred males within a breeding aggregation,

perform better than males in a place learning task (Liu and Burmeister 2017). Although these findings support the adaptive specialization hypothesis, less is known about the cognitive mechanism(s) that produce observed sex differences in cognitive tasks.

Maze tasks are often used to investigate sex differences in cognition, but performance in mazes can be influenced by a variety of factors, including motivation (Gaulin and Wartell 1990), training parameters (Gatto et al. 2017; Gingins et al. 2018), and the types of cues used (Sandstrom et al. 1998; Roof and Stein 1999; Torres et al. 2014). For example, male and female rats are both capable of solving the Morris water maze, but males tend to rely on geometric cues when available, whereas females tend to rely on landmarks (Torres et al. 2014). Anurans are able to use both egocentric cues

(e.g., turning left or right) (Schmajuk et al. 1980; Brattstrom 1990; Daneri et al. 2011) and allocentric cues (e.g., visual cues such as landmarks) (Jenkin and Laberge 2010; Daneri et al. 2011; Sotelo et al. 2015; Liu et al. 2016) to learn a variety of maze tasks. In a study of cue preference during place learning, Argentine toads tend to use egocentric cues, rather than provided visual cues, to learn the task (Daneri et al. 2011). A similar preference for egocentric cues over allocentric cues exists in other vertebrates (hamster: Teroni et al. 1987; rat: Cohen et al. 1990; mouse: Alyan and Jander 1994). In a previous study, release orientation of túngara frogs was always perpendicular to the long axis of the maze and varied in a pseudo-random fashion to eliminate the potential for subjects to use turn direction (an egocentric cue) to remember the location of the maze exit (Liu and Burmeister 2017). However, for trials in which the release orientation was the same for 2 consecutive trials, males performed as well as females, indicating that they were remembering their last turn direction for those trials (Liu and Burmeister 2017). Given that egocentric cue preference is probably a conserved trait, the sex difference in place learning in túngara frogs may have emerged from a differential ability to switch from egocentric cues to the provided visual cues, rather than a differential ability to use visual cues to remember locations in space.

We set out to test whether the sex difference in place learning observed by Liu and Burmeister (2017) would generalize to other training parameters with different cues available. To do so, we replicated the earlier study with a few modifications. We switched location of the provided visual cue so that it was in a different location each trial; thus, only the provided visual cue indicated the correct location. We released frogs into the maze with random orientation. Thus, unlike in the earlier study (Liu and Burmeister 2017), turn direction could never be used to remember the correct location in the maze. Finally, we trained frogs using 3 trials per day (instead of 2), which may improve learning. If the sex difference observed by Liu and Burmeister (2017) reflects a general difference in the ability to learn allocentric cues, females should outperform males in this study.

Materials and Methods

Animals

We began with 24 sexually mature túngara frogs (12 males and 12 females) that were naïve to any behavioral experiments. These animals were 2–6 generations derived from native populations collected in Panama. We maintained animals in conditions similar to their native tropical climate at 23°C–25°C and 65–75% relative humidity (RH) at a 12:12 light:dark cycle (lights on at 08:00 h). They were separated into 12 same-sex terraria; we distinguished between 2 frogs in 1 terrarium by applying colored nontoxic nail polish (Piggy Paint Nail Polish: Piggy Paint, LLC) to the frogs' dorsal sides. We fed the frogs fruit flies dusted with calcium and vitamins 3 times per week. The University of North Carolina's Institution for Animal Use and Care Committee approved all procedures (protocol 14-026).

Apparatus

Frogs were trained in a 2-arm maze described previously (Liu et al. 2016). The maze was constructed from white fiberboard, was covered by glass during training to prevent escape (Figure 1), and was surrounded by a white curtain to block external cues. At the end of each maze arm, there was a door cued by either red or yellow; the materials used were the same as previously described (Liu and Burmeister 2017). In Liu and Burmeister (2017), a preference test
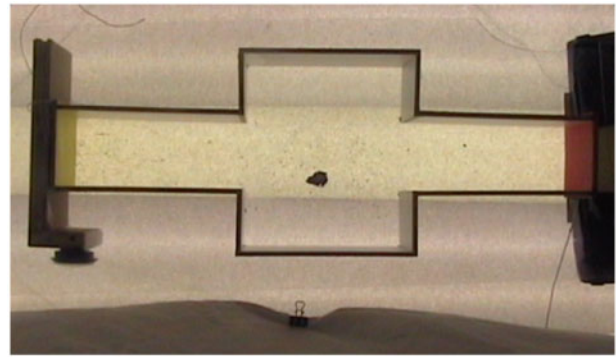

Figure 1. The 2-arm maze used to test the frogs' ability to associate a maze exit with access to shelters and return to the home cage. Subjects were released in a central chamber (18 cm × 21 cm) and allowed to choose 1 of 2 arms (each 19 cm × 8 cm) to reach an exit. The end of each arm was blocked by a door (9 cm × 9 cm), one of which could be opened to reveal shelters.

indicated no initial preference for red or yellow, and the red door was rewarded with return to the home cage for all frogs.

We placed the maze on a light table covered with a laboratory soaker paper to create a bright and shadow-free environment in the maze (Figure 1). We increased the temperature and decreased the humidity by placing space heaters just outside the maze. Together, this created a bright, hot, and dry environment (~35°C and 10–20% RH) inside the maze which motivated the frogs to find the exit to gain access to the shelters and return to the home cage. For the first 7 days of training, the heaters were located behind the shelters outside each door which may have inhibited the frogs from exiting the maze; on Day 8, we moved the heaters outside the central chamber for the remainder of the trials. We replaced the laboratory soaker paper after each day of trials.

Procedure

Frogs were acclimated to the environment of the maze before acquisition trials began with 2 trials per day over 2 consecutive days. During acclimation, both red and yellow doors to the maze were removed, leaving both arms open to a covered shelter. The frog was released in the middle of the starting chamber at a random orientation and given 3 min to exit the maze through either arm and enter a shelter, at which time the frog would be transported back to its home terrarium. If the frog had not exited the maze after 3 min, a small straw would be used to nudge the frogs toward the nearest exit.

On the day following the acclimation, the frogs began training with 3 trials per day. Frogs were trained for 10 consecutive days and trained in the same order (Subjects 1–24 consecutively) for all 3 trials, with an intertrial interval of ~45 min. Sexes and door color were interspersed during training. During acquisition, the incorrect door was held immobile by a brick whereas the correct door could be opened to reveal 3 shelters. Half of the frogs (6 males and 6 females) were trained to exit through the yellow door and half through the red door.

The maze itself remained in the same orientation on the light table for the duration of acquisition, but door location was switched every trial in relation to the maze and any other potential allocentric cues. This procedure ensured that, to solve the maze, the frogs would have to use the local cue (door color), as no other allocentric cue would reliably indicate the exit.

To begin a trial, frogs were corralled from their home environment into a small cup, which was held upside down with a small

piece of poster board on the bottom. The entire cup was then placed in the center chamber and lifted up at the beginning of each trial to release the frog. Due to the unknown position of the frog at the time the cup was lifted, the frog always began the trial at a random orientation in relation to the maze exits. Frogs were given 3 min to exit the maze. We defined a choice as approaching within 2 cm of a door. We scored the trial as correct if the first choice was the correct door. If the frog succeeded in exiting the maze within 3 min, we recorded the trial as complete whether or not the correct door was approached first. In incomplete trials, we opened the correct door and nudged the frog in the correct direction if it had not moved toward the exit 30 s after the conclusion of the trial. In every trial, after a frog entered one of the shelters at the end of the correct arm of the maze, it was transported back to its home cage.

Statistical analysis

We noticed that on the first trial of the experiment, before learning occurred, there was a preference for choosing the red door: out of 19 completed trials, the frogs chose red 15 times (chi-square goodness of fit $= 6.4$, $P = 0.01$). Thus, for subsequent analyses, we focused on yellow-rewarded frogs ($n = 12$). In addition, we used the tendency to approach the yellow door (4/19; 21.1%) as the null hypothesis against which we tested for learning. Using a z-score calculator, we determined that, for a sample size of 12, choosing the yellow door 53.6% of the time differs significantly from 21.1% ($P = 0.049$).

Performance in this task could improve through a number of mechanisms, including learning to associate the cue with the exit and learning the nature of the task—in this case, to find an exit. Thus, we examined the percentage of completed trials across training using a repeated measure analysis of variance (ANOVA) after arcsine transformation of the data, as they are proportions.

To test for an increase in percentage of correct choices in completed trials across days, we used a linear trend analysis in all 12 frogs rewarded with the yellow door. We also used a 1-tailed paired t-test to examine whether color preference (percentage of trials in which red was chosen) changed by comparing preference on Days 1 and 30. To test whether females and males differed in their response to training, we used repeated measures ANOVA (sex $\times$ day) on percent correct in completed trials. Because the percentage of correct data are proportional, we arcsine transformed them before
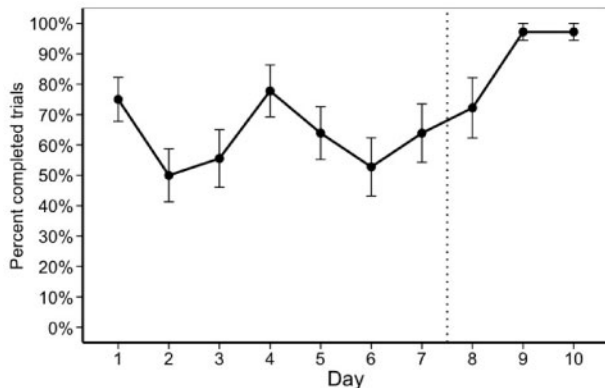
statistical analysis. We used R Studio (Version 1.1.442, R Foundation for Statistical Computing, Vienna, Austria) for all statistical analyses.

Results

The percentage of completed trials increased from 63% in the first 2 days of training to 97% in the last 2 days of training (Figure 2; day: $F_{11,99} = 5.62$, $P < 0.001$), reflecting an increasing familiarity with the task. As a group, the frogs showed an increase in the percentage of correct choices (linear trend: $F_{1,11} = 14.0$, $P = 0.003$), approaching, but not reaching, the criterion for learning (Figure 3A). While the group did not meet the learning criterion, 4 individuals (2 females and 2 males) exceeded the threshold on the last day of training. In addition, there was a decreased preference for the red door on the last day of training compared with the first (Figure 4; $t_{11} = 2.08$, $P = 0.03$). However, there was no evidence of a sex difference in learning (Figure 3B; sex $\times$ day: $F_{9,198} = 1.11$, $P = 0.36$).

Discussion

We found that the apparent female advantage reported by Liu and Burmeister (2017) was no longer evident when animals were trained under the present conditions, suggesting that the sex difference observed by Liu and Burmeister (2017) does not reflect a general sex difference in the ability to use allocentric cues to remember locations in space.
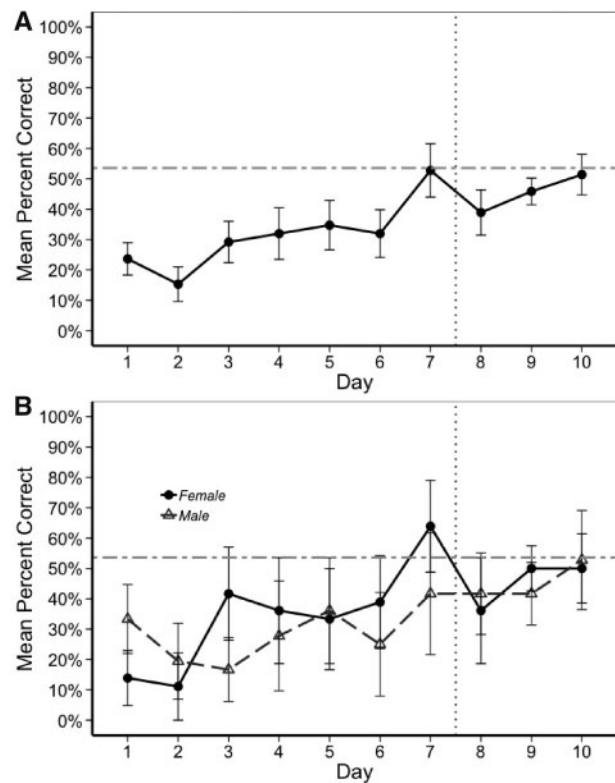

Figure 3. The percentage of correct choice (mean ± SE) across training days for all frogs rewarded by the yellow door ($n = 12$) **(A)** and for females and males separately **(B)**. The criterion for learning is denoted by the horizontal gray dashed lines at $y = 54\%$. The point at which heaters were moved from behind the exits of the maze to the sides of the maze is denoted by the vertical dotted lines.

Figure 2. The percentage of completed trials (mean ± SE) increased with training ($n = 12$). The point at which heaters were moved from behind the exits of the maze to the sides of the maze is denoted by the vertical dotted line.
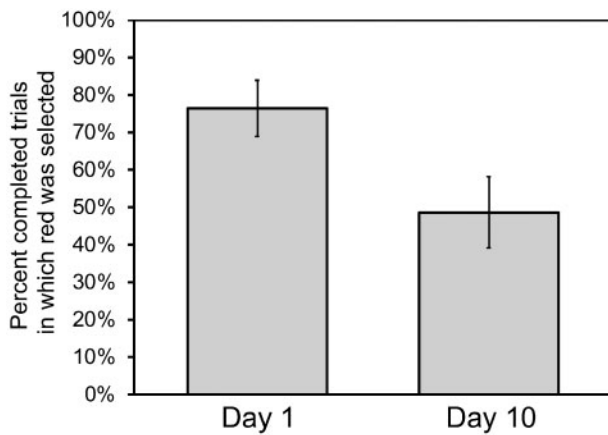
Figure 4. The percentage of trials in which the red door was chosen on the first and last day of training in frogs rewarded by the yellow door ($n = 12$).

While we did not find evidence of a sex difference in learning in this study, our ability to draw strong conclusions is limited by the more modest evidence of learning compared with Liu and Burmeister (2017) which may have been complicated by the preference for the red door. In anurans, blue is preferred in lower light settings whereas red and orange are preferred in brighter light (Jaeger and Hailman 1976). Our results are consistent with this finding, as we tested frogs on a light table resulting in relatively high light intensity. The lack of color preference observed in Liu and Burmeister (2017) may be because they tested the frogs under lower light levels. It is worth noting that in the earlier study (Liu and Burmeister 2017), all frogs were trained to the red door and all females were able to learn the task. And while no color preference was detected in that study, there could have been a bias toward learning to associate red with reward. Such biases toward learning one color over another have been documented in other species (e.g., Colwill et al. 2005). The fact that, in this study, we only considered frogs trained to the yellow door may be 1 reason that fewer frogs learned the task than in Liu and Burmeister (2017).

During navigation, animals receive cues generated by the body (egocentric cues) and the external environment (allocentric cues) (Burgess 2006). Naturally, those cues would not conflict with each other. However, if these 2 types of cues contradict each other during place learning, animals show a hierarchy to use the 2 types of cue (Redish 1999). Typically, egocentric cues receive priority. This phenomenon has been found in mouse (Alyan and Jander 1994), rat (Cohen et al. 1990), hamster (Teroni et al. 1987), and Argentine toad (Daneri et al. 2011). Liu and Burmeister (2017) found that male túngara frogs used egocentric cues even when allocentric cues were more reliable, resulting in an apparent female advantage during place learning. One modification in this study was that egocentric cues were dissociated from reward, which may have released the males from their dependence on egocentric cues. Although further studies are necessary, the data are consistent with the hypothesis that males and females have similar place learning abilities but that males rely on egocentric cues when they are available and fail to switch to allocentric cues. It is possible that allocentric cues are more useful to females as they recall locations of potential mates. This is broadly similar to cases in which species that rely on spatial memory prefer to use distal cues to code relationships between cues and a goal (Clayton and Krebs 1994). The processes that drive such species differences may be similar to those that drive cognitive specialization between sexes.

Sex differences in cognition have been described through a number of specific behavioral tasks (Gaulin and Fitzgerald 1989; Guigueno et al. 2014). Although differential performance is often taken as evidence of better or worse cognitive ability, their underlying behavioral mechanisms have less often been carefully examined. We studied túngara frogs, in which females perform better than males in a place learning task (Liu and Burmeister 2017) and showed that the apparent differential learning ability depends on the experimental context. Our study is a reminder that sex differences in cognitive ability may not always result from differences in ability *per se* but from other underlying cognitive processes.

Acknowledgments

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