Visual discrimination learning in the fire-bellied toad *Bombina orientalis*

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This study explored the visual discrimination learning ability of fire-bellied toads (*Bombina orientalis*). Two groups of toads were trained in a simultaneous visual discrimination task involving video footage of either black crickets on a white background (*black-cricket toads*) or white crickets on a black background (*white-cricket toads*). Fifteen widely spaced acquisition trials were followed by 12 reversal trials. Successful learning was observed by decreased incorrect snapping and reduced latency to snap at the correct stimulus (S+) during acquisition; however, white-cricket toads executed significantly more incorrect snaps than did black-cricket toads. Both groups of toads could master the reversal task as measured by latency to snap at S+, but not as measured by the proportion of incorrect snaps. Despite the stronger potency of the black-cricket stimulus, the results showed that toads can learn a simultaneous discrimination task and a reversal of its contingency. This elaborate form of learning appears to be conserved among vertebrates.

Associative learning involving visual discrimination between two alternatives presented simultaneously has been demonstrated in many vertebrate and invertebrate animals (Bussey et al., 2008; Colwill, Raymond, Ferreira, & Escudero, 2005; Giachetti, Orsini, Fogassi, Francesconi, & Musumeci, 1985; Giurfa, 2004; Gonzalez, Behrend, & Bitterman, 1967; Kelber, 2002; Lucchetta, Bernstein, Théry, Lazzari, & Desouhant, 2008; Mackintosh & Mackintosh, 1964; Nissani, Hoefler-Nissani, Lay, & Htun, 2005; Reiner & Powers, 1983). However, evidence of simultaneous visual discrimination learning in an adult amphibian is still lacking, as is evidence of reversal learning on such a task. Despite the observation that larval newts can discriminate arbitrary visual symbols when they are paired with food, adult newts cannot learn that task (Hershkowitz & Samuel, 1973). Demonstrations of reversal learning in amphibians have been limited to maze studies using water as a reinforcer (Brattstrom, 1990; Ellins, Cramer, & Martin, 1982; Schmajuk, Segura, & Reborada, 1980). Reversal of a nonspatial visual discrimination has been demonstrated in different species of fish and reptiles (Behrend, Domesick, & Bitterman, 1965; Colwill et al., 2005; Day, Crews, & Wilczynski, 1999; Gonzalez et al., 1967; Woodward, Schoel, & Bitterman, 1971); therefore, one could expect that this ability is also present in amphibians.

Despite past difficulties encountered when trying to use amphibians as subjects in conditioning experiments (see Thompson & Boice, 1975), it has since been shown that toads can be conditioned under certain circumstances (Brattstrom, 1990; Muzio, Ruetti, & Papini, 2006; Muzio, Segura, & Papini, 1992; Papini, Muzio, & Segura, 1995;

Schmajuk et al., 1980; Suboski, 1992). The fire-bellied toad *Bombina orientalis* was chosen in the present study in order to explore the possibility that it exhibits simultaneous visual discrimination learning. This species is easy to handle and will readily orient and snap at prey cues displayed on a computer screen, allowing for reward manipulation. Other advantages of using *B. orientalis* include the relative ease of rearing a colony in captivity and recent progress in the description of the anatomy of its forebrain, which has enabled evaluation of brain structure–function relationships (Endepols, Mühlenbrock-Lenter, Roth, & Walkowiak, 2006; Endepols, Roden, Luksch, Dicke, & Walkowiak, 2004; Laberge, Mühlenbrock-Lenter, Dicke, & Roth, 2008; Mühlenbrock-Lenter, Endepols, Roth, & Walkowiak, 2005; Roth, Laberge, Mühlenbrock-Lenter, & Grunwald, 2007; Roth, Mühlenbrock-Lenter, Grunwald, & Laberge, 2004).

There are currently between six and eight species recognized in the genus *Bombina* (Yu, Yang, Zhang, & Rao, 2007; Zheng, Fu, & Li, 2009). These toads are known for the *unken reflex*, a display that involves arching of the back and extremities, which exposes their orange underside. This response is thought to be an antipredator display of aposematic coloration signaling that the toads are poisonous (Choi, Lee, & Ricklefs, 1999; Löhner, 1919). Indeed, their dorsal skin secretions contain bombesin toxins (Nagalla et al., 1996). They represent one of the oldest living frog lineages, dating back to at least the Jurassic period (Yu et al., 2007). *B. orientalis* inhabits forests and meadows in temperate northeast Asia. Its ecology, however, has been little studied. The only formal report on foraging ecology in *B. orientalis* was made on the forest-dwelling

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toads in the Russian portion of their distribution range (Korotkova, 1978). It showed that these toads are generalist predators of small invertebrates, with a preference for lepidopteran larvae; however, the main food in their diet varies with prey abundance. Furthermore, adult males display intraspecific oophagy (consumption of eggs) during the breeding season, when they remain in the same area for a few months (Marshall, Doyle, & Kaplan, 1990).

As a generalist predator, *B. orientalis* is likely exposed to different prey types during its lifetime, allowing the opportunity to develop preferences or aversions. Discrimination learning could enable optimization of foraging preferences. Furthermore, it was recently proposed, on the basis of work in *B. orientalis*, that amphibians and mammals share important similarities in the brain pathways implicated in the regulation of motivated behavior (Laberge & Roth, 2007). In light of the demonstration of this type of learning in many other animals, therefore, we investigated the possibility that the amphibian *B. orientalis* can discriminate between two distinct visual prey stimuli on the basis of selective reward.

METHOD

Experimental Animals

Fifty-six adult fire-bellied toads (*B. orientalis*) were purchased from either Global Exotic Pets (Kitchener, ON) or Port Credit Pets (Mississauga, ON). The toads were housed at the Hagen Aqualab of the University of Guelph in a room kept at a temperature of 21°C during the day and at a temperature of 17°C at night under a 12:12-h light:dark photoperiod. The toads were housed in glass tanks (37 cm long $\times$ 22 cm wide $\times$ 25 cm high) with gravel substrate, broken clay pots, and flat stones for cover. A water dish was also included to ensure constant access to water. Before the experiment began, the toads were fed crickets (*Acheta domesticus*) lightly dusted with calcium and vitamin powder until satiety. During the experiment, the toads received food only in the form of a reward, except when a toad did not perform the task successfully. In the latter case, the toads that did not receive a food reward during trials were fed a cricket on a separate day outside of the experimental context. Individual toads were identified on the basis of their unique body color patterns.

Video Stimulus

Approximately 10 crickets were placed in a clear plastic tube against a white background. They were filmed in high definition using a Canon VIXIA HV20 camcorder (Canon, Tokyo, Japan). A 5-min video clip was selected, and a negative version was created using Adobe CS3 Premiere Pro (Adobe Systems, San Jose, CA). This resulted in two 5-min clips of the same moving cricket stimulus in two different color configurations: the original dark crickets on a white background and the inverted clip of white crickets on a dark background. Both clips were standard VGA size (640 $\times$ 480) at 30 frames per second. The two video clips were displayed side by side in two panels on a computer screen (LP 1965 19-in. LCD, Hewlett-Packard, Palo Alto, CA), and the side on which the black or white cricket was presented was randomized (Figure 1). The screen was mounted to the wall, with its bottom part resting on a table.

Experimental Procedures

The experimental context was a square platform (40 $\times$ 40 cm) made of gray PVC cut to fit between the edges of the computer screen and remain flush against it. The platform and computer were on top of a larger table. There were no walls around this experimental context; therefore, the toads faced only the computer screen while the experimenter stood behind. Each toad was first tested for its response toward the video clip of prey. If a toad displayed snapping, the experimental trials began. Snapping consisted of direct contact with a stimulus displayed on the computer screen, and the identity of the contacted stimulus was always unambiguous (i.e., toads did not snap between or beside the cricket displays). Unresponsive toads were subjected to a pretraining procedure, to familiarize them with the experimental context. During this procedure, individual toads were placed on the platform facing the computer screen, which was turned off, and were fed a cricket. These toads were later tested again for their response to the prey stimulus. All toads were snapping or at least fixating on the stimulus after a maximum of three pretraining trials. Food rewards consisted of one cricket held with tweezers in front of the toad's line of sight. The toads generally accepted food presented in this way at the first trial.

At the beginning of each trial, the toad was placed on the square platform roughly 4 in. away from the screen, facing the latter in a position equivalent to the middle of the two stimuli panels. At this time, the computer screen was completely black. The experimental trials began with the display of the clips, which could be stopped by the experimenter at any time by a touch of the keyboard that returned the screen to a complete black display. The trial was stopped when a toad snapped at the correct stimulus, or when the maximal trial

Figure 1. Photograph of the testing arena. A fire-bellied toad is seen attending to the right panel of two cricket stimuli presented simultaneously, side by side on the computer screen.

duration of 5 min (300 sec) was reached. A stopwatch was used to record the latency to a correct snap and to keep track of the maximal trial duration.

Two different groups were used. In one group (*black-cricket toads*, $n = 28$), toads were trained to snap at black crickets, and they received a food reward only if they snapped at the black-cricket stimulus panel. Each toad was rewarded with one cricket (unaltered) immediately after each successful snap. In the other group (*white-cricket toads*, $n = 28$), toads were trained to snap at white crickets, and they received a food reward only if they snapped at the white-cricket stimulus panel. Each toad was rewarded immediately with one "whitened" cricket. The crickets are naturally dark, so in order to color the crickets white, a calcium and vitamin powder was dusted over them before they were used as reward. If a toad snapped only at the inappropriate cricket stimulus, the screen remained on until the 5-min clip ended, and no food reward was given. The number of snaps toward both the correct stimulus (S+) and the incorrect stimulus (S-) were recorded. In some cases, the toads did not snap at any stimulus during the 300-sec trial. These occurrences were termed *no-response trials*. The experiment took place between 10:00 a.m. and 1:00 p.m., and subsequent trials were separated by 3 or 4 days. Acquisition data for a first batch of 26 toads was collected between January and March 2009; reversal for this batch of toads began in May. A second batch of 30 toads underwent acquisition between May and June 2009 and underwent reversal between June and July. The latter batch thus differed from the first one, because it did not experience a pause before reversal.

Statistical Analysis

A two-tailed chi-square test was used to evaluate whether black-cricket and white-cricket toads differed in regard to the proportion of no-response trials and the proportion of incorrect snaps (number of incorrect snaps divided by total number of snaps). The remaining data were analyzed using nonparametric statistics, since they were not normally distributed. A two-tailed Wilcoxon signed-rank test was used to determine whether the proportion of incorrect snaps was greater than 0 at each trial within each group in the early phase of the experiment and following reversal. The value of 0 was chosen because it was achieved by the end of acquisition in each group. The same test was used to analyze data on the total number of snaps, but in that case it tested whether the value at each trial was greater than 1, which is the minimum number of snaps an animal could achieve in a successful trial and which was observed by the end of acquisition in each group. The latency data was analyzed using a two-tailed Mann-Whitney test comparing values at Trial 1 with each subsequent trial within each group. All statistical tests were performed using Prism 4 (GraphPad Software, La Jolla, CA) with α set at .05.

RESULTS

Seven toads (4 white-cricket, 3 black-cricket) were rejected because they did not acquire the task. They snapped only a few times, or not at all, at the cricket stimuli before all snapping activity stopped. A further 3 toads (all white-cricket) died after 4 acquisition trials and were also rejected from the analysis, whereas 2 toads (1 white-cricket, 1 black-cricket) that died after 7 and 11 acquisition trials were included. Of the 46 toads included for data analysis, 15 (11 black-cricket, 4 white-cricket) snapped only at S+ over all acquisition trials; they made a correct choice by chance and maintained their response. These data were analyzed separately.

Acquisition of the Visual Discrimination

The proportion of no-response trials (no snaps at either stimulus) was significantly higher in white-cricket

toads (18.5%) than in black-cricket toads (8.4%) [$\chi^2(1, N = 690) = 15.6, p < .0001$]. The proportion of incorrect snaps was also significantly higher in the white-cricket toads (36.2%, compared with 8.7% in black-cricket toads) [$\chi^2(1, N = 728) = 79.8, p < .0001$]. Among toads that made mistakes, there was no difference in snapping behavior between toads of the first and second batches; therefore, the two batches were combined for further analysis (proportion of incorrect snaps among all trials in white-cricket toads, Mann-Whitney $U = 3,597, n_1 = 66, n_2 = 122, p = .18$; total number of snaps in the same toads, $U = 3,972, n_1 = 66, n_2 = 122, p = .86$). The two batches of black-cricket toads were not compared, because only 3 of them were obtained from the first batch, preventing meaningful statistical calculations.

A decrease in the proportion of incorrect snaps was observed in both black-cricket and white-cricket toads over the 15 acquisition trials (Figure 2A). This variable was not statistically different from 0 at Trial 3 [$W(13) = 3, p > .05$] and all subsequent trials in black-cricket toads, whereas the same was observed at Trial 9 and all subsequent trials in white-cricket toads [$W(9) = 1, p > .05$]. Note that 8 out of the 14 toads that made mistakes in the black-cricket group displayed incorrect snaps at only the first trial and then snapped only at S+ following the first rewarded trial. Such a fast acquisition was not seen in white-cricket toads. Figure 2B shows that the total number of snaps during acquisition was not significantly higher than 1 at Trial 3 [$W(13) = 3, p > .05$] and all subsequent trials in black-cricket toads, whereas this occurred at Trial 7 [$W(14) = 10, p > .05$] and Trials 9–15 in white-cricket toads. The latency to snap at S+ was significantly reduced at Trial 4 ($U = 20.5, n_1 = n_2 = 12, p = .0032$) and all subsequent trials in black-cricket toads, whereas it was reduced at Trials 5 ($U = 37, n_1 = 14, n_2 = 11, p = .0305$), 6, and 8–15 in white-cricket toads (Figure 2C).

Figure 3 shows the data on latency to snap at S+ for all toads that never made an incorrect snap during acquisition trials. Statistical analysis showed that latency was reduced at Trials 7 ($U = 35.5, n_1 = 12, n_2 = 14, p = .0134$), 8, and 10–15.

Reversal

All toads that had acquired the discrimination task as well as those that never made a mistake during the acquisition period underwent reversal of the rewarded stimulus for a total of 12 trials. This reversal of contingency began at Trial 16. Before reversal, an attempt was made to see whether the toads (5 black-cricket toads and 5 white-cricket toads, chosen randomly in the first batch) could snap at a white or black panel alone without a prey stimulus, in order to see whether screen color rather than cricket color induced snapping. The two panels displayed side by side without the moving cricket stimuli did not induce any snapping, despite the fact that the toads had already acquired the visual discrimination on the basis of prey cues moving in these panels. This experience changed later performance during reversal in the black-cricket toads (all trials, proportion of incorrect snaps, $U = 4,441, n_1 = 48$,

Figure 2. (A) Proportion of incorrect snaps, (B) total number of snaps, and (C) latency to snap at the correct stimulus (S+) during acquisition of the visual discrimination (Trials 1–15) and following reversal of S+ (Trials 16–27). One group of toads is identified by inverted triangles ($n = 14$ during acquisition, and $n = 20$ after reversal in A and C; $n = 14$ in B), the other by circles ($n = 17$ during acquisition and $n = 14$ after reversal in A and C; $n = 17$ in B). The color of the symbol (i.e., black or white) represents the color of S+. Time of reversal is indicated by the dotted vertical line between Trials 15 and 16. NS indicates trials at which the proportion of incorrect snaps is not significantly different from 0 (in panel A) or from 1 (in panel B) following the Wilcoxon signed-rank test both during the course of acquisition and following reversal. The asterisk in panel C indicates a significant difference from Trial 1 following the Mann–Whitney test. NS and asterisks with arrows pointing to the right represent continuous blocks of statistically nonsignificant (proportion and number) or significant (latency) trials within the acquisition or reversal periods. Statistical symbols relating to inverted triangles are below, whereas those relating to circles are above. Data are means $\pm$ SEM.

Figure 3. Latency to snap at the correct stimulus during acquisition of the visual discrimination in toads that never made a mistake. Data (means $\pm$ SEM) of 11 toads rewarded for the black stimulus and 4 toads rewarded for the white stimulus were combined. The asterisk indicates a significant difference from Trial 1 following the Mann-Whitney test. The asterisk with an arrow pointing to the right represents a continuous block of statistically significant trials.

$n_2 = 234, p = .02$; total number of snaps, $U = 4,224, n_1 = 48, n_2 = 234, p = .006$) but not in white-cricket toads (all trials, proportion of incorrect snaps, $U = 3,582, n_1 = 48, n_2 = 159, p = .45$; total number of snaps, $U = 3,850, n_1 = 48, n_2 = 168, p = .56$). Black-cricket toads exposed to the empty panels had a smaller proportion of incorrect snaps and a lower total number of snaps after reversal compared with toads never exposed to the empty panels; therefore, all animals exposed to the empty panels were excluded from further analysis of reversal data.

Since there was a longer delay before the onset of reversal trials in the first batch of toads, data from the two batches were compared. When all trials were considered, there was no difference in the proportion of incorrect snaps ($U = 5,444, n_1 = 84, n_2 = 132, p = .79$) or in total number of snaps ($U = 5,412, n_1 = 84, n_2 = 132, p = .72$) between the two batches of toads switched to a black S+. The same was true of toads switched to a white S+ (proportion of incorrect snaps, $U = 9,190, n_1 = 115, n_2 = 167, p = .53$; total number of snaps, $U = 8,390, n_1 = 115, n_2 = 167, p = .07$). The two batches of toads were thus grouped together for further analysis of reversal data.

At the first trial following reversal, the proportion of incorrect snaps and the total number of snaps were both higher in black-cricket toads when compared with the first acquisition trial (proportion, $U = 20.5, n_1 = 10, n_2 = 12, p = .009$; number, $U = 20, n_1 = 12, n_2 = 10, p = .007$). This was not the case in white-cricket toads (proportion, $U = 43.5, n_1 = 12, n_2 = 11, p = .16$; number, $U = 46, n_1 = 12, n_2 = 11, p = .19$), and this was not the case for both groups with latency to snap at S+ (black-cricket toads, $U = 19, n_1 = 10, n_2 = 6, p = .26$; white-cricket toads, $U = 32.5, n_1 = 10, n_2 = 11, p = .12$). One can also observe in the reversal data charted in Figure 2 that the black-cricket toads switched to a white S+ did not

decrease their snapping behavior to the level seen at the end of acquisition. This occurred despite a clear trend in reduction of the proportion of incorrect snaps and of the total number of snaps. This is unlike the white-cricket toads that were switched to a black S+, which returned to a low proportion of incorrect snaps at Trial 20 [$W(14) = 3, p > .05$] and subsequent trials and to a low total number of snaps at Trial 19 [$W(11) = 3, p > .05$] and subsequent trials. However, the data on latency to snap at S+ show that both groups of toads diminished their latency significantly during the reversal period when compared with the first acquisition trial (Figure 2C). This reduction was seen at Trial 17 ($U = 18, n_1 = 10, n_2 = 12, p = .006$) and subsequent trials in white-cricket toads switched to a black S+ and at Trial 24 ($U = 47.5, n_1 = 10, n_2 = 19, p = .03$) and subsequent trials in black-cricket toads switched to a white S+.

The data for toads that never made an incorrect snap were included in the reversal trials charted in Figure 2. In light of the difference in snapping behavior in one group following reversal, however, these data for 12 toads that never made a mistake during acquisition were explored separately (3 of these toads were rejected after taking part in the panel test). Figure 4 shows that these black-cricket toads that were switched to a white S+ also displayed a greater total number of snaps in the early reversal trials than did the white-cricket toads that were switched to a black S+. Furthermore, the total number of snaps in these black-cricket toads that were switched to a white S+ was not significantly different from 1 at the last reversal trial [$W(9) = 10, p > .05$]. The sample size for white-cricket toads that were switched to a black S+ was too small to

Figure 4. Total number of snaps after reversal of the correct stimulus (S+) in toads that never made a mistake. Open inverted triangles represent toads switched from black S+ to white S+ ($n = 9$). Filled circles represent toads switched from white S+ to black S+ ($n = 3$). NS indicates a trial that is not significantly different from 1 following the Wilcoxon signed-rank test. Sample size was too low to perform this test in toads switched from white S+ to black S+. The subset of toads shown black crickets on a white background that never made a mistake following a single reward is also charted for comparison purposes (gray inverted triangles, $n = 7$). Data are means $\pm$ SEM.

perform statistics. Additional data for 7 black-cricket toads that never made a mistake following a single reward are also shown in Figure 4 (mistakes restricted to first trial, gray symbols). Their total number of snaps was similar to that for the black-cricket toads that never made a mistake (all trials, $U = 3,764$, $n_1 = 83$, $n_2 = 104$, $p = .13$).

DISCUSSION

The present study revealed that fire-bellied toads are able to learn a simultaneous visual discrimination task. In both black-cricket and white-cricket toads, incorrect snapping behavior was absent, and the latency to snap at S+ was greatly reduced by the end of the 15 acquisition trials. The toads could also master a reversal of the original rewarded stimulus contingency as measured by a decrease in latency to snap at S+ in both groups. After reversal, however, the proportion of incorrect snaps and the total number of snaps did not diminish below their original value at Trial 1 in toads that were switched from a black-cricket to a white-cricket stimulus. Furthermore, the higher numbers of incorrect snaps and no-response trials during acquisition in white-cricket toads suggest that the latter stimulus was less potent than the black-cricket stimulus.

Motivation

The presence of no-response trials is attributed to a lack of motivation. In an attempt to ensure that the toads were adequately hungry for each consecutive trial, the trials were separated by 3 to 4 days. Although this precaution was taken, many toads exhibited no-response trials between successful trials. One potential reason for these occurrences was that after a few successful trials, in which a food reward was given, hunger could have decreased. Another potential reason for the lack of motivation to snap could have been the amount of food the toads received prior to the experiment. The first batch of 26 toads was fed to satiety once a week for two months before experimentation, whereas the second batch of toads was used shortly after arrival. In fact, all 7 toads rejected from the experiment because they did not acquire the task were from the first batch of 26 toads. Pretraining trials were used only for toads of this batch. The toads in the second batch were highly motivated to snap, probably because of a higher level of hunger.

Color Bias

The results show a difference in potency to elicit snapping between the black-cricket and white-cricket stimuli. This difference could be due to a variety of reasons. Prey preference in animals is determined by a mixture of innate and experience-dependent mechanisms. For example, newborn snakes show an initial preference for prey cues that represent the normal diet of their species, but this preference is easily modifiable by experience (Burghardt, 1967, 1971; Fuchs & Burghardt, 1971). It is unclear whether the tendency to snap more at the black-cricket stimulus that was observed in the present study has any innate component. The toads could be innately prone to

snap at black crickets, or they could be innately prone to associate food reward with dark prey stimuli. Further investigation of that issue will have to determine whether naive toads never exposed to crickets also show a bias toward black crickets.

The toads used here could have learned to prefer dark crickets because they were fed such prey prior to the experiment; however, freshly dusted crickets are lightly colored and are readily accepted as food. This is also the case with newly shed crickets that are white. The exposure to dark prey prior to the experiment was thus not complete. Despite the latter fact, the higher prevalence of dark crickets fed prior to the experiment could explain the color bias observed here. It would be interesting to see whether such a color bias would persist in toads raised on a diet of lightly colored prey (e.g., wax worms).

Another explanation for the color bias is a potential difference in palatability between the crickets whitened with calcium and vitamin powder and the unaltered dark crickets. Indeed, humans report that calcium has a mineral taste that is moderately bitter, and physiological work in amphibians supports the existence of a specific calcium taste mechanism (reviewed in Tordoff, 2001). A mild aversion to the taste of whitened crickets could have led to a diminished reward value, which in turn would have led to slower learning after experience of that reward, as observed here. However, amphibians held in captivity are known to suffer calcium deficiency as a result of the low quality of their food. This could have increased the preference for the whitened crickets. Furthermore, the powder was not a novel taste, since the toads were fed whitened crickets prior to the experimental trials, and when a food reward was given, powdered or not, the toads always consumed it. If whitened crickets are aversive at all, the preference for black crickets should increase with accumulated experience of both prey types, something that could be investigated using naive toads.

There is also the possibility that toads have a seasonal preference in prey color. Ewert and Siefert (1974) found that in the summer, toads (*Bufo bufo*) prefer to snap at white prey dummies, but that this preference undergoes a reversal at the onset of winter, when toads prefer black dummies. In the present experiment, however, toads were tested in two different seasons, the first batch in winter and the second in summer, and there was no obvious change in color preference across seasons.

The discrimination of white prey against a black background could have been more difficult than the reverse. More difficult visual discriminations have been shown to delay the increase in correct responses, as was observed here when black-cricket and white-cricket toads were compared (see Mackintosh, 1974, p. 148). This issue is not likely to apply to the present study, since the stimuli were the same for both groups and we used an inverted image to create the white-cricket stimulus. The potential discrepancy in the display of the two stimuli thus seems restricted to brightness. It would be interesting to see whether the toads can transfer their learning to the presentation of real stimuli without further training (i.e., preference for live black or live white crickets). The latter could alleviate the fear that one of the two stimuli is not perceived adequately

(D'Eath, 1998). Obviously, an explanation of the observed color bias will require further investigation.

Comparative Aspects

Previous studies of simultaneous visual discriminations involved training sessions with multiple trials during the same day, repeated on different days (Bussey et al., 2008; Colwill et al., 2005; Giachetti et al., 1985; Gonzalez et al., 1967; Nissani et al., 2005; Reiner & Powers, 1983). Amphibians typically have lower energy requirements and feed less often than the animals used in the above learning studies; thus, the toads used here were not given multiple food rewards during one experimental day. As a result, the learning schedule differed slightly from what has been used in other animals, but it is expected that a single trial per day should diminish the chance of learning compared with the use of training sessions, which can only strengthen the present observations. For the purpose of comparison, it would be interesting to test whether massed trials result in a similar learning profile in toads. This might be accomplished by using multiple smaller rewards with hungry toads.

Some toads never made any mistakes during the acquisition trials. Mackintosh (1974, p. 192) and Bitterman (1975) described how rats and pigeons can sometimes display systematic responses to the same position in discrimination situations. Considering that the position of the visual stimuli was randomized in the present experiment, it appears that some toads were systematically responding to the same stimulus. The presence of a subset of animals within a population displaying a systematic behavioral strategy in discrimination situations could be a common feature among vertebrates.

The present investigation used a response requirement similar to that in a study in rats by Morton, Skillings, Bussey, and Saksida (2006), with the exception that the visual stimuli they used were not biologically relevant. Learning about nonbiologically relevant cues and learning about biologically relevant cues are known to proceed differently (Domjan, 2005). Since it appears that toads will not snap at inert objects, as shown by the panel test prior to reversal, it would be interesting to observe the behavior of rats or birds toward displays of prey cues (e.g., crickets, worms). Conditioning protocols using computer displays of biologically relevant cues could allow comparisons between related and phylogenetically distant species.

Conclusions

The limited number of studies of learning in amphibians prevents in-depth comparisons with other animals. More such work is required to allow for a better understanding of the evolution of learning and cognition in vertebrates. Of interest here is the recent observation that the brain pathways implicated in the regulation of motivated behavior share important similarities in amphibians and mammals (Laberge & Roth, 2007). By investigating the brain pathways of simple vertebrates, such as amphibians, a more solid understanding of the complex pathways regulating behavior in mammals could be developed.

AUTHOR NOTE

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