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Environmental enrichment impacts discrimination between familiar and unfamiliar human odours in snakes (*Pantherophis guttata*)

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ABSTRACT

Environmental enrichment has been found to significantly influence the cognitive abilities of a variety of mammalian and avian species, with effects ranging from positive to negative, however, these effects have been little studied in reptiles. This is problematic given their popularity as pets and the wide variation in their care. Therefore, the aim of this study was to examine how exposure to environmental enrichment affected discrimination between familiar and unfamiliar humans in a popular species of pet reptile, the corn snake. Snakes (n = 11) were individually housed for four weeks in either an enriched or standard environment before we tested their discrimination between familiar and unfamiliar odours of humans (familiar handler vs. unfamiliar stranger). The snakes were then swapped into the other housing treatment (either enriched to standard, or standard to enriched) for a further four weeks before being tested again. In the discrimination tests, the snakes were simultaneously presented with the odours of a familiar and unfamiliar human within a test arena, and the time spent in close proximity to either stimulus was recorded. We found that after being housed in the enriched enclosures the snakes spent significantly more time investigating the unfamiliar human odour, suggesting successful discrimination of the handlers, and an attraction to novelty. In contrast, snakes housed in the standard enclosures did not discriminate between the two odours despite exploring the stimuli for the same overall amount of time. Therefore, this study demonstrates that corn snakes can recognize the odour of familiar humans; however, this was only observed in the enriched group, suggesting that the absence of environmental enrichment may interfere with discrimination in this task. We recommended that enclosures incorporate enrichment in order to promote good welfare.

1. Introduction

Environmental enrichment is a concept that describes beneficial changes to the environment of an animal by encouraging the expression of natural behaviours (Young et al., 2020; Wiedenmayer, 1996). When implemented appropriately, there is strong evidence to suggest that it reduces stress and improves welfare (Coulton et al., 1997; Leal-Galicia et al., 2008). Enrichment may also have numerous cognitive benefits in mammals and birds, improving performance in tasks requiring spatial exploration (Jones and Waddington, 1992), visual discrimination and memory retention (Leal-Galicia et al., 2008), amongst others. However, the effects of environmental enrichment can prove complicated because it appears to vary when the duration (Leger et al., 2015) and/or specific elements of the enrichment are altered. For example, domestic chicks (Gallus gallus domesticus) showed higher levels of spatial exploration and a reduced fear of humans when exposed to environmental enrichment,

however they only showed the latter when exposed solely to handling (Jones and Waddington, 1992). This implies that specific types of enrichment can cause a generalized habituation to novelty, while others may not.

In contrast to mammals and birds, reptile species have received little attention in this context. This is partly due to the perception that reptiles adapt well to minimalistic enclosures (Warwick, 1990), but also the misconception that they are sluggish and their behaviour is largely controlled by innate drives (Wilkinson and Glass, 2021). There is now evidence of impressive cognitive abilities in this group (see Matsubara et al., 2017, for a review), including complex social learning (e.g. Wilkinson et al., 2010; Kis et al., 2015), spatial learning (e.g. Mueller-Paul et al., 2012, 2014), visual discrimination and long-term memory retention (e.g. Soldati et al., 2017). With their cognitive abilities being comparable to those of many mammals and birds (Wilkinson and Huber, 2012), alongside the increasing popularity of reptiles as pets (Burman

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et al., 2016), there is a clear need to further our understanding of the impact that environmental enrichment has upon the cognition and welfare of reptiles.

Recently, some studies have explored the impact of enrichment on reptile behaviour, with contrasting results. For instance, the addition of chemosensory enrichment (the odour of another conspecific) has been shown to impact the behaviour of wall lizards (Podarcis liolepis). The enriched animals were faster to habituate to novel arenas, and displayed significantly less stress-related behaviours (e.g. escape attempts; Londoño et al., 2018). In contrast, the addition of climbing enrichment (height = 14 cm) to a barren enclosure had no significant effects on eastern fence lizard (Sceloporus undulatus) behaviour (Rosier and Langkilde, 2011). Though it should be noted that the climbing enrichment may not have been enriching given that they can frequent heights of ten feet in the wild (Kennedy, 1958). Aside from lizards, rat snakes (Pantherophis obsoletus) were found to benefit from the inclusion of various housing enrichments (e.g. elevated coconut hide, damp moss hide, branch and substrate) and the addition of live prey. The rat snakes displayed distinct behavioural profiles depending on housing condition as observed by their cumulative behaviour in three tasks, showing an overall change in behaviour when exposed to environmental enrichment (Almli and Burghardt, 2006).

One aspect of cognition that is particularly relevant to animals living in captivity is their ability to interact with humans. Direct interactions with humans can be stressful (Broom and Johnson, 1993) especially when those individuals are unfamiliar (Jones and Waddington, 1992). Environmental enrichment may improve an animal's ability to process cues by habituating them to a broad range of stimuli, which decreases fear responses and promotes the recognition of subtle differences between cues (Leal-Galicia et al., 2008). If animals are able to distinguish between familiar handlers and strangers, it may improve welfare by giving them greater choice and control over their environment (i.e. to approach/avoid); it is also likely to improve the human-animal bond by encouraging preferential interaction.

The aim of this work was therefore to investigate whether there was an impact of environmental enrichment on performance in a discrimination task by a popular pet reptile, the corn snake (*Pantherophis guttata*), when exposed to the odour of familiar and unfamiliar humans. The odour domain was used because of snakes' reliance on chemoreception (Gilingham and Clark, 1981) and evidence of successful odour discriminations by this group (Burger, 1989; Pernetta et al., 2009).

2. Methods

2.1. Subjects

Eleven corn snakes (*Pantherophis guttata*) that ranged in length, 80-125~cm (average = $104.18\pm3.9~cm$) were used in this study. They were loaned from a reptile shelter, and thus had a varied range of backgrounds and experiences. Individual histories, sex and age were unknown. Upon arrival, they were placed into one of two housing conditions (see below). Each snake was housed individually, in plastic coated vivariums ($82cm\times38~cm\times32~cm$; $18~^{\circ}C-30~^{\circ}C$ thermal gradient, 7am-7pm photoperiod) that contained UV lighting and a heat lamp. All the snakes were handled regularly, except during shedding and after feeding. The snakes were fed suitably sized mice once a week.

2.2. Housing

The snakes were housed in either a standard or enriched enclosure for four weeks before being given a series of behavioural tests. They were then switched into the other housing condition (e.g. standard to enriched, or enriched to standard). After four weeks in the new condition, they received a second round of testing using the same cognitive tests.

2.2.1. Standard condition

Enclosures contained sheets of newspaper as substrate, a small water dish and a single rock hide (Fig. 1).

2.2.2. Enriched condition

Enclosures contained shredded aspen as a substrate, a large water bowl that allowed submersion, a climbing board, branch, and three different types of hides ranging in humidity and elevation (moss, rock, hanging coconut; Fig. 1).

2.3. Stimuli

The odour samples were collected from three male and three female human participants. Two of the humans (one male and one female) were the experimenters and they acted as the familiar humans. The unfamiliar humans (two male and two female) had never met, or interacted with, the snakes, and they were matched for sex and age (+/-3) years) with the experimenters. The familiar humans regularly handled the snakes (five times a week except during shedding), and had consistent contact with each of the snakes over the experimental period. They did not have contact with other animal species. Based on previous research, this exposure was considered to be sufficient for the animals to perceive the handlers to be familiar (e.g. Burman and Mendl, 2006; Stephan et al., 2012). The odour samples were collected the day before testing; the participants firmly held rectangular shaped cotton pads ($10 \text{ cm} \times 20 \text{ cm}$) rolled up within a closed fist for ten minutes. A total of 64 pads (32 of each stimulus type) were collected throughout the experiment and cut into squares (10 cm × 10 cm) for single use application. Disposable gloves were worn by the experimenter when cutting up the pads. The disposable gloves were changed for every stimulus. The pads were then grouped by stimulus and placed into airtight Ziploc bags to retain their odours overnight (Settle et al., 1994; Pernetta et al., 2009).

2.4. Testing procedure

The snakes received two test trials that took place in a temperature-controlled room (24–27 °C). A test followed exposure to each housing condition and was run in one of two visually distinct arenas (83 cm \times 83 cm, arenas differed in terms of wall and floor covering). Prior to the odour test, the snakes were habituated to the test arena and to scentless cotton squares (habituation to the latter took place in their home enclosure). The arenas were cleaned (diluted Safe4 solution) between each trial, and the order of arena use was counterbalanced across subjects.

Before the trial began, two scented cotton squares (10 cm \times 10 cm) belonging to a familiar and matched unfamiliar person were placed in the centre of neighbouring quadrants on markers approximately 50 cm from where the snake was placed (Fig. 2). The positions of the stimuli were counterbalanced across individuals and trials to avoid bias. The floor of the arena was marked with quadrants (40 cm \times 40 cm) to allow assessment of the snakes' position. Proximity was dictated by head placement; the snake was deemed to be in a certain quadrant once the tip of its snout passed over the line. At the start of each trial, the snake was placed on a pre-set marker that was equidistant from each stimulus. This was done by the experimenter whose scent was not being used as a stimulus for that specific trial. The snakes were placed in a coiled position with their head facing forward. The trials began after the experimenters were out view, this was approximately 20 s after the snake was placed in the arena (Hoehfurtner et al., 2021), this pause was used in order to minimize the effect of handling on the data - most snakes remained stationary during this time. Each trial lasted ten minutes, a duration found suitable in previous research (Chiszar and Carter, 1975; Hoehfurtner et al., 2021). After each trial, the snake was returned to its enclosure. All trials were filmed from an overhead camera for subsequent analysis.

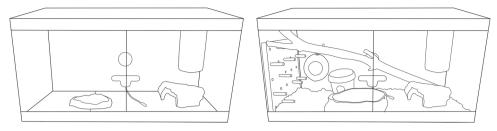


Fig. 1. Diagram of Standard (left) and Enriched (right) enclosures. Taken from Hoehfurtner et al., submitted.

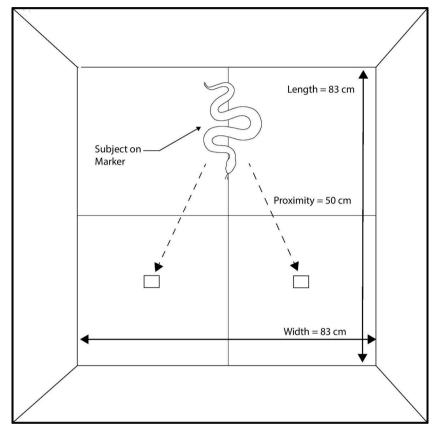


Fig. 2. A diagram of the test arena setup.

2.5. Video and data analysis

Inter-rater reliability was calculated using the Intraclass Correlation Coefficient (ICC) via SPSS based on single measures, a consistency-agreement and a 2-way mixed effects model. The reliability between raters was found to be excellent (ICC = 0.992 with 95 % Confidence Interval = 0.986–0.996). The amount of time the snakes spent in quadrants containing the test stimuli was analysed. BORIS software (Friard and Gamba, 2016) was used to analyse the videos and SPSS (IBM SPSS Statistics 23) was used for the statistical analyses. A Shapiro-Wilks test confirmed normality of the data, therefore a repeated-measures General Linear Model (GLM) with Environment (standard/enriched) and Scent (familiar/unfamiliar) as within-subject factors was used. Paired samples t-tests were used to identify any post-hoc differences.

3. Results

The repeated-measures GLM revealed that there was no main effect of either Environment (F(1,10) = 0.008, p = 0.929) or Scent (F(1,10) = 0.448, p = 0.518). There was, however, a significant Environment*Scent interaction, (F(1,10) = 5.006, p = 0.049). A post-hoc paired t-test

revealed that enriched snakes spent significantly more time inspecting the unfamiliar human odour (M = 0.346 ± 0.034) than the familiar (M = 0.219 ± 0.031 ; t(10)= -2.978, p = 0.014; Fig. 3). There was no overall difference in willingness to approach the stimuli between the housing conditions (t(10) = 0.30215, p = 0.7682). Please see supplementary

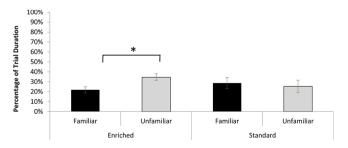


Fig. 3. The enriched snakes (n = 11) spent significantly more time within the quadrant containing the odour of the unfamiliar individual (M = 0.346 \pm 0.034) compared to the familiar individual (M = 0.219 \pm 0.031), t(10)= -2.978, p = 0.014, while standard snakes showed no significant difference between the time spent in odour quadrants Data are presented as means +/- std. error.

information for individual data.

4. Discussion

This study investigated the impact that environmental enrichment has on performance of a learned task in snakes by examining their ability to discriminate between the odours of familiar and unfamiliar humans. We found that snakes were able to discriminate between humans based on familiarity, however, they only did so when housed in an enriched environment. The effect was clear despite there being no overall difference in their willingness to explore the stimuli. This finding suggests that the presence of enrichment may be a key factor in allowing snakes to make, or display, this sort of odour discrimination. Further, the work represents the first evidence that snakes can discriminate between the odour of people and that differences in the housing environment impacts performance of this task.

Almli and Burghardt (2006) found that prolonged exposure to enriched housing along with live feeding, resulted in different behavioural profiles between two groups of rat snakes. However, their study did not find a significant difference in learning between the groups. Our work demonstrates that, when living in an enriched environment without live feeding, corn snakes were able to discriminate between the odour of familiar and unfamiliar humans but did not do so when living in standard housing conditions. This difference in behaviour, as a result of enrichment, has also been observed in mammals (Leger et al., 2015) and birds (Jones and Waddington, 1992) and suggests that the presence of appropriate enrichment may foster a positive relationship with the environment that results in improved discriminative abilities. This could occur because the enriched snakes were routinely exposed to a larger variety of novel and complex stimuli in their enclosures which may have provided more opportunity for learning about familiarity, while reducing their fear response to novelty, and focusing more on the specific differences between stimuli. The observed willingness of the enriched snakes to approach the novel odour may be related to increased risk tolerance and as a result of the enrichment which potentially led to increased neophilia when living in this housing condition. There is strong evidence to support the effect of enrichment on neophilia in both mammals (Leger et al., 2015) and birds (Jones and Waddington, 1992) and there is some evidence of this in snakes (Hoehfurtner et al., 2021). Thus, the attraction to novelty (the unfamiliar human) that was in observed in this experiment suggests that living in an enriched environment promoted the expression of natural behaviour in the snakes, indicating the need for permanent, enriched housing in this group.

Interestingly, the difference in performance was observed both when animals were housed in the enriched condition before the standard condition and vice versa (when housed in the standard condition before being housed in the enriched condition), suggesting that the effect of enrichment may be transitory and may depend on the snake's current, or most recent, housing conditions. This transient nature of the effect of enrichment is also observed in mammals (Leger et al., 2015) and highlights the need for permanent, enriched housing for reptiles. This is particularly important given the rapid benefit seen from these housing conditions, with differences observed after just a few weeks. It is, however, important that the rapid change observed is not used as evidence to support keeping reptiles in unenriched environments temporarily. There is strong evidence to suggest that reptiles have substantial long-term memory (Soldati et al., 2017; Mueller-Paul et al., 2014) and that they can generalise experience in one environment to a different one (Mueller-Paul et al., 2014), therefore it is important to continually provide enriched housing for this group.

It has also been suggested that handling can be viewed as a form of enrichment in mammals and birds (though see below; Jones and Waddington, 1992; Fernández-Teruel et al., 2002). In our experiment the standard snakes received identical handling experience to the enriched snakes, however, under the test conditions, they did not discriminate between the odours. This suggests that, if enriching, handling is not

enriching enough to elicit the discrimination observed in the enriched snakes. Thus, we recommend that captive snakes receive housing enrichment in addition to handling.

The nature of this experiment is particularly relevant for animals living in captivity as interactions with humans can be stressful (Broom and Johnson, 1993), and enclosure conditions can vary substantially. If snakes can learn to distinguish between familiar and unfamiliar humans, it would allow them to respond appropriately to these individuals, giving them greater control over and choice in their environment and interactions, this is likely to minimise stress and improve welfare (Jones and Waddington, 1992). For example, those animals that have positive interactions with humans (e.g. good handling) are, in turn, faster to approach people in subsequent interactions, suggesting an improvement in welfare (Gonyou et al., 1986; Gouveia and Hurst, 2013). It is also likely to impact upon the human-animal bond (Saito and Shinozuka, 2013), as it allows snakes to learn about their handlers or owners. As such, the associated benefits of positive human-animal interactions are not only for the animals themselves, but also for the human caregivers (e.g. Hemsworth et al., 2000).

In conclusion, our work demonstrates that enriched environments alter the performance of snakes in a discrimination task. Under current guidelines, snakes can be kept in relatively barren environments and our work reveals that this is likely to impact upon their stimulus perception and subsequently their welfare. We therefore recommend that pet owners should keep pet snakes in enriched conditions. Future studies are essential to investigate how enrichment impacts other aspects of behaviour, learning and cognition of snakes and other reptiles, particularly given their increasing popularity as pets.

Declaration of Competing Interest

The authors declare no conflict of interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.applanim.2021.10 5278.

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