Using Negative Reinforcement to Promote Non-Agonistic Behavior in a Green Iguana (*Iguana iguana)*

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Abstract

Green iguanas (*Iguana iguana*) are a large, long-lived reptile well-equipped to defend themselves against predators and territorial conspecifics. These increasingly common household pets often respond to human caretakers with anti-predator behaviors including hissing and tail whipping. Current green iguana literature recommends long-term socialization training without any well-defined protocols. Animal behaviorists recommend positive reinforcement training whenever possible; however, negative reinforcement may be a suitable alternative when working with frightened or aggressive animals. Removal of aversive human presence contingent on the subject animal's behavior has been shown to promote calm and friendly behaviors in domestic cats (*Felis catus*), domestic dogs (*Canus lupus familiaris*), and petting zoo sheep (*Ovis aries*). Negative reinforcement research has primarily focused on domestic mammals, but high-level learning capabilities have been demonstrated in a variety of reptile species. Thus, the present study aimed to apply negative reinforcement training to promote non-agonistic behaviors in a captive green iguana. Following 12 days of negative reinforcement training, an increase in non-agonistic behaviors were observed with a decrease in the overall number of agonistic displays and types of agonistic behavior presented. This study indicates that the previously described negative reinforcement protocols for domestic mammals also promote non-agonistic behaviors in a captive pet green iguana utilizing a naturally occurring and unavoidable stimulus to promote behavior change in a least intrusive manner.

Keywords: animal training; green iguana; negative reinforcement; reptile

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Introduction

The Green Iguana (*Iguana iguana*)

Wild green iguanas (Iguana iguana) have been collected for decades, over a million each year in the 1990s alone (Kaplan, 1991), becoming one of the most popular reptile species in the pet trade (National Geographic, 2011). They are now readily available in pet stores, where juveniles can be purchased for less than 100 USD (Meshaka, et al., 2004). When properly cared for, green iguanas can live up to 20 years, growing 6-7 feet in length and weighing an average of 15 pounds (Dowling, 2008). However, owing to the lack of accurate information available to caretakers, most green iguanas do not survive their first year or are surrendered to rescue organizations due to prohibitive cost or aggressive behaviors (National Geographic, 2011). Sometimes they are released or escape back into the wild (Meshaka, et al., 2004). Unlike cats and dogs, green iguanas are not a domesticated species. Frequently, wild caught individuals are directly sold as household pets and therefore they behave similarly, if not entirely, like their wild counterparts (Kaplan, 1991). Thus, a thorough understanding of their evolutionary history is vital for the proper care and welfare of the non-domesticated green iguana.

Green iguanas are large lizards with striped tails (Figure 1) serving to camouflage them in the rainforest canopies of Central and South America where they naturally occur in the wild (Greene et al., 1978; Kaplan, 1991). As agile climbers, they are typically found in the canopy eating or resting, but they are also skilled swimmers, readily dropping from the trees to the water below (Swanson, 2004). Furthermore, females are excellent diggers capable of excavating burrows up to three feet deep where they subsequently deposit and bury their eggs (Kaplan, 2000). Herbivorous animals, green iguanas have sharp teeth for ripping pieces off the plant and swallowing it without chewing (Distel & Veazey, 1982; Kaplan, 1991). Predators of green iguanas include crocodiles, other large reptiles (e.g., *Boa constrictor*), large birds (e.g., double-toothed kite *Harpagus bidentatus*), and wild cats (Greene et al., 1978). Green iguanas are also a good source of meat and are hunted by humans (Swanson, 2004). Keen visual acuity and hearing enables green iguanas to respond to the movement and sounds of other animals. Living in social groups, particularly among juveniles or females at nesting sites, provides additional safety for iguanas (Greene et al., 1978; Kaplan, 2000; Swanson, 2004). Additionally, rapid agility allows for flight in trees, in ground burrows, or under water. Laterally compressed bodies, fully extended dewlaps, and wide-open mouths warn predators or conspecifics of impending defensive maneuvers including scratching and tail whipping (Greene et al., 1978; Swanson, 2004). A list of commonly observed behaviors and their descriptions is provided in Table 1 (Distel & Veazey, 1982). As a non-domesticated species with a wide variety of predators, it is common to see similar anti-predator behavior from captive green iguanas in the presence of their human caretakers (Kaplan, 1991). Conversely, many green iguanas live peacefully as pets should their human caretakers provide appropriate care and training (Kaplan, 2000).

Green Iguana Behavior

As many wild-caught green iguanas are placed into captive settings, including homes as companion animals, it is to be expected that they may display anti-predator behaviors. However, it is also likely that many caretakers do not properly understand these behaviors and will be quick to label them as abnormal behaviors displayed by “jerks”, “rude” or “insane” animals, or otherwise dangerous and aggressive animals. Such labels are explanatory fictions, hypothetical explanations of observed circumstances without any understanding, and are often circular in nature contributing no information to the situation (Cooper et al., 2019).

Naturally, misunderstanding green iguana behavior might cause caregivers to put their green iguana in a situation that compromises its welfare. For example, captive green iguanas are typically confined to smaller environments as pet owners view them as sedentary animals not needing additional space. Rather, wild green iguanas can be quite active within their home ranges and hypoactivity in captive green iguanas may indicate disease or injury (Warwick et al., 2013). Additionally, as ectotherms, green iguanas are behavioral thermoregulators, moving to and from different locations as needed to access the precise temperature required. However, they have also been known to display voluntary hyperthermia and hypothermia presumably after bouts of stress or disease, respectively (Warwick et al., 2013). Thus, appropriate understanding of behavior may also provide early insight into medical issues (Hellmuth et al., 2012). Improper husbandry and misinterpretation of behavior can result in reduced welfare leading to illnesses, stunted growth, or a shortened lifespan for the animal (Kaplan, 1991). Such misunderstanding may also lead to anti-predator, defensive, or fear-based behaviors displayed by green iguanas (e.g., tail whipping) being deemed aggressive, and in turn, resulting in the animal being surrendered to a rescue group or intentionally released into the wild (Repass, 2020; Meshaka, et al., 2004). Broom (2021) defines aggression as, "[a]n act or threat of action, directed by one individual towards another, with the intention of disadvantaging that other individual by actually or potentially causing injury, pain or fear." However, as described above, it is possible for behaviors to be misidentified as aggression. Agonistic behavior is more encompassing and is defined as, "[a]ny behaviour associated with threat, attack or defence. It includes features of behaviour involving escape or passivity, as well as aggression" (Broom 2021). Table 2 divides the behaviors of green iguanas into agonistic and non-agonistic categories.

Benefits of Training

Captive green iguanas frequently display agonistic behaviors, particularly during breeding season (Kaplan, 2000). Although there are numerous handbooks available for green iguana caretakers (e.g., Mahmoud (n.d.) and Repass (2020)), most provide rudimentary information with little to no support in systematic scientific research; nevertheless, most suggest daily gentle handling to permit the animal to acclimate to their human caretaker allowing for a positive human-animal relationship (Mahmoud, n.d.; Repass, 2020). However, the handbooks rarely mention a specific method of gentle handling to facilitate acclimation. As non-domesticated animals, "[i]guanas rarely become tame and socialized on their own," (Kaplan, 2000). In captivity, interactions with humans are inevitable and can have profound effects, both positive and negative, on the welfare of the animal (Mellor et al., 2020). Captive reptiles acclimated to human presence are less likely to experience “flight-related stress and self-injury” and have been reported to interact and bond with specific caretakers (Warwick & Steedman, 1995). One potential response to handling stress is autotomizing or dropping their tail. This self-amputating process allows the muscles and vertebrae in the tail to sever from the body and can leave the animal vulnerable to infections in the open wound (Kaplan, 2000). Thus, desensitizing captive green iguanas to human presence and handling can minimize stress and prevent injuries (Hellmuth et al., 2012).

Outside of daily husbandry, green iguanas also must be handled during veterinary care. Traditionally, most veterinary care must be done under physical restraint or anesthesia which poses a variety of safety concerns to both the animal and the human caretaking staff (Young & Cipreste, 2004). Risks include the animal having an adverse reaction to the chemicals used for anesthesia and/or displaying fear-based behaviors potentially resulting in the animal biting, scratching, or severely injuring the caretaker (Hellmuth et al., 2012; Young & Cipreste, 2004). Safety concerns during medical procedures are not limited to owned non-domesticated species. Zoos also face these concerns. Thus, zoological facilities train a variety of species to ensure staff can work safely around large, potentially dangerous animals and to facilitate medical care of the animals (Blowers & Rudy, 2021; Kincaid, 2021). This type of training, referred to as cooperative care, involves training animals to actively participate in their husbandry and care rather than simply tolerating it. There has been a wide variety of research in cooperative care. For example, Callealta et al. (2020) demonstrated the benefits of training African lions (*Panthera leo*) to voluntarily participate in the collection of vaginal swabs and blood samples. With positive reinforcement, the animals were trained to lie on a platform for up to 20 minutes while the samples were collected. Throughout the training, the caretakers remained safely outside of the animal enclosures and demonstrated an alternative minimally invasive medical procedure with less psychological and physiological stress for the lions than traditional methods using anesthesia. Implementing training, especially positive reinforcement training, can lead to improved welfare and positive animal-caretaker relationships (Brando, 2012). Rault et al. (2020) cite numerous benefits of a positive human-animal relationship including lower risk of injury, animal learning, increased stress resilience, and a positive welfare state.

Training can be conducted through reinforcement, whereby the frequency of a targeted behavior increases, or punishment, whereby the frequency of a targeted behavior decreases (Cooper et al., 2019). Furthermore, positive reinforcement or punishment indicates a stimulus has been added to the environment, while negative reinforcement or punishment indicates a stimulus has been removed (Cooper et al., 2019). Research has shown that punishment is often coupled with increased aggression, fear, and avoidance behaviors (Cooper et al., 2019; Friedman et al., 2021). Additionally, punishment seeks to eliminate behaviors teaching the learner what not to do, but reinforcement of alternative behaviors increases the learner's behavioral repertoire and teaches them what to do instead (Cooper et al., 2019). Punishment does not provide opportunities for the animal to learn alternative behaviors and, therefore, it has been suggested that such an approach should be avoided (Friedman et al., 2021). Instead, Friedman et al. (2021) recommend a hierarchy for behavior change elevating least intrusive methods over more aversive ones, thus suggesting positive reinforcement as the ethical standard above all other techniques, including extinction and negative reinforcement, with positive punishment holding the lowest tier.

Positive Reinforcement

Cooper et al. (2019) define positive reinforcement as occurring "when a response is followed immediately by the presentation of a stimulus change that increases the future occurrence of similar responses." Since the behavior temporally closest to the delivery of reinforcement is the behavior that will be strengthened, it is vital that reinforcement is provided immediately as any number of other behaviors other than the target behavior may occur during a delay (Cooper et al., 2019). Appetitive stimuli for use in reinforcement can come in many forms including edible reinforcers, sensory reinforcers, tangible reinforcers, and social reinforcers. Examples of the use of positive reinforcement with exotic and wild animals abound in the literature and include species such as bears (Spears, 2022), chimpanzees (Green et al., 2021), cockatoos (Maillard & Sugar, 2022), sloths (Johnson, 2021), meerkats (Simon, 2022), rheas (Prinsen & Ferrell, 2022), tigers (Townzen & Sides, 2021), wolverines (Keller & Wright, 2022), cranes (Zolinksi, 2023), and hippopotamus (Bliss et al., 2023).

Positive reinforcement can be used in training procedures such as shaping. Fernandez and Dorey (2021) successfully demonstrated the use of shaping, whereby successive approximations to an end goal are differentially reinforced, in training an African crested porcupine (*Hystrix cristata*). The end goal was for the porcupine to touch and hold position on a target for 30 seconds to allow the animal to be placed in various locations while zookeepers cleaned the enclosure. The training was divided into 10 steps, or approximations. The porcupine received a food reward after each correct response but did not move to the next approximation until nine out of 10 trials were successfully completed for three consecutive sessions. Additionally, a decrease in undesired behaviors, such as chewing and moving away, was demonstrated and the authors concluded this systematic approach to training was conducted more efficiently than previously used methods.

Through positive reinforcement training, common marmosets (*Callithrix jacchus*) were trained for voluntary weight and urine collection (McKinley et al., 2003). As with the porcupine study, a shaping method was used with pairs of marmosets receiving a food reward for completing each approximation towards the end goal. This procedure reduced data collection time and potential health costs compared to traditional collection methods. Additionally, a richer human-animal relationship was reported because of the training. During this study, there were four instances of aggression observed when one marmoset tried to steal the food reward of the other; however, the authors found that the aggression could be eliminated by ensuring training was conducted only after the animals had already been fed and by providing a less preferred food item (McKinley et al., 2003). This use of positive reinforcement training is a one example of how many zoological facilities are promoting cooperative care of their animals where the animals voluntarily participate in their own veterinary care (Brando & Norman, 2023).

A zoo-housed olive baboon (*Papio hamadryas anubis*) was reported to exhibit self-injurious behaviors including biting her limbs and pulling out her hair. A functional analysis was conducted in which antecedents and consequences are experimentally arranged to observe the separate effects on the problem behavior that needs to be addressed (Cooper et al., 2019). The research team was able to determine that the self-injurious behavior was being positively reinforced by zookeeper attention. By providing the baboon with attention for an alternative appropriate behavior, lip-smacking, the self-injurious behavior decreased, and the lip-smacking behavior increased (Dorey et al., 2009). This is an example of differential reinforcement where behaviors other than the target behavior are reinforced (Cooper et al., 2019) and also demonstrates positive reinforcement with the use of a social reward, rather than an edible one.

Furthermore, a study comparing positive reinforcement training of horses (*Equus caballus*) to more traditional negative reinforcement showed the positive reinforcement training to be highly efficient (Sankey et al., 2010). In each trial, the experimenter gave a vocal order for the horse to move backward and then took a step towards it. If the horse took a step back within 10 s, the experimenter provided a food reward. The training was successfully accomplished in 15 min and the effects were still visible five months later. Also, the body posture of the horses was examined during the training; agonistic behaviors, such as laid-back ears or head tossing, were not seen, but were observed in horses trained by other methods. Additionally, after the training the horses showed an increased interest in humans by actively seeking contact not only with the trainers, but with unfamiliar humans as well (Sankey et al., 2010).

Negative Reinforcement

Desensitization is a technique commonly recommended with fearful cats and dogs (Rentfro, 2012; Katz & Rosales-Ruiz, 2022). This technique gradually exposes an animal to a stimulus that may be perceived as new or otherwise frightening. Hellmuth et al. (2012) describe habituation as passive desensitization, where the animal gets used to the stimulus on their own without active involvement from a trainer or any pairing with reinforcement. They contrast this with counter conditioning (active desensitization) in which the stimulus is paired with reinforcement (Hellmuth et al., 2012). To prevent increased fear responses, desensitization must be conducted very slowly. In many cases the end-result benefits of such an approach may arrive too late, such as in a medical emergency when quick actions are needed but will likely bring additional stress from unfamiliar veterinary staff and equipment. Conversely, other training techniques, such as negative reinforcement, might be a necessary precursor for increasing alternative suitable behaviors in an animal whose current behavior is potentially maintained through negative reinforcement. Negative reinforcement increases the frequency of a targeted behavior by removing an aversive stimulus (Cooper et al., 2019). Frequently, the use of negative reinforcement induces ethical concerns, as for example Sankey et al. (2010) note that it, “implies delivering an aversive (i.e. unpleasant) stimulus.” However, if the human caretaker is already perceived as aversive, removing the human can provide an escape contingency for the animal, thereby negatively reinforcing the behavior by creating increased distance to the human caretaker (Smith & Iwata, 2019). The use of negative reinforcement through the removal of a human from the situation has been used to increase calm and friendly behaviors in domestic cats, domestic dogs, and petting zoo sheep (Rentfro, 2012; Katz & Rosales-Ruiz, 2022; Fernandez, 2020).

In her Master’s thesis, Rentfro (2012) developed a Fearful to Friendly (F2F) approach to increase adoptability of feral kittens. These kittens frequently display fearful and aggressive behaviors suitable for survival in the wild, but which prevent them from meeting adoption criteria at animal shelters. Kittens in this study were housed separately in enclosures within a larger room. The protocol required a series of incrementally progressive training steps to reduce the distance between the experimenter and the kittens. Initial steps included walking into the room with the kitten enclosure up to predetermined locations and then leaving the room contingent on calm, desirable behaviors emitted by the kitten. Conversely, when the kitten emitted undesirable behaviors, the experimenter stopped approaching the enclosure and waited for the undesirable behavior to lessen, upon which the experimenter turned and exited the room. Once the experimenter could successfully enter the room and walk up to the kitten's enclosure, the protocol progressed through a series of steps that allowed for the experimenter to open the enclosure door and later to extend an arm towards the kitten. Throughout the study, the goal was to minimize agonistic behaviors from the kittens by keeping them below their fear threshold. Upon successful completion of these steps, the focus of the training switched to positive reinforcement with brushing and petting. At the conclusion of the study, all four kittens displayed more desirable behaviors than undesirable behaviors and overall training was conducted in less than 48 hours.

Similarly, the use of negative reinforcement has been demonstrated in domestic dogs. Snider's Master's thesis (2007) demonstrated differential negative reinforcement of alternative behaviors in three aggressive dogs. Here the author was able to build a repertoire of safe alternative behaviors to replace aggressive behaviors; this differs from traditional approaches of desensitization or counterconditioning which rely on reduced levels of responding. These traditional approaches are time-consuming, often taking months or years, and do not promote other behaviors. However, the use of negative reinforcement was conducted in a timelier manner, with several dogs completing treatment in under two hours, while also demonstrating long-lasting effects with improved behavior continuing to be reported over a year after treatment.

Moreover, Katz and Rosales-Ruiz (2022) were able to use negative reinforcement to shape an approach behavior in fearful shelter dogs. Fearful behaviors, such as remaining at the back of the kennel and avoiding interactions with humans, increased the dog's length of stay (Protopopova et al., 2014; Weiss et al., 2012). Using negative reinforcement, all three dogs in the study successfully learned to approach the front of the cage and interact with a novel person which later resulted in adoption in each case. This was accomplished after only one training session further demonstrating the efficiency and efficacy of negative reinforcement training.

Additionally, Fernandez (2020) demonstrated the use of negative reinforcement in fearful petting zoo sheep effectively decreasing the distance between the trainer and sheep, such that the trainer could then maintain these behaviors through positive reinforcement. By removing the naturally occurring, unavoidable aversive stimulus (the human), the trainers increased the sheep's behavioral repertoire instead of using traditional methods which rely on exposure to aversive stimuli to reduce already occurring behaviors. These methods suggest the potential to quickly reduce undesirable behaviors in other species displaying fearful or aggressive behaviors (Rentfro, 2012; Snider, 2007).

Gaps in Reptile Research and Training

The majority of research conducted in animal learning has focused on birds and mammals. However, there has been some research on reptiles that has indicated high-level learning capabilities. For example, rapid problem solving has been demonstrated in black-throated monitor lizards (*Varanus albigularis albigularis;* Manrod et al., 2008). These animals were presented with a novel task apparatus where food was placed inside a transparent tube over three presentations. All individuals successfully retrieved the food within the first 10 min of the first trial. Also, mean latency to retrieve the food over successive presentations and ineffective strategies, such as shaking the tube, decreased.

Leal and Powell (2012) demonstrated the behavioral flexibility of emerald anoles (*Anolis evermanni*) across a variety of tasks. The lizards were first presented with the task of dislodging a cover that was placed over a well containing food. If the animals were successful in the first task, they were then presented with two wells, one of which contained food covered by the original cover and the other empty and covered with a differently colored top. Finally, the lizards progressed to a task where the food was switched and placed under the new lid. The tests indicated the lizards were able to solve novel motor tasks via multiple strategies, visual discrimination of blue- and yellow-colored discs, and reversal learning, respectively. As noted by the authors, behavioral flexibility is commonly believed to be restricted to social species, such as mammals and birds, that live in complex environments, with varied food sources, and unpredictable resources; their research indicates a need to reconsider ectothermic cognitive abilities.

Leopard geckos (*Eublepharis macularius*), described as an asocial reptile, demonstrated gaze-following abilities (Simpson & O'Hara, 2019). This ability to gather information by following the gaze of another individual is most often seen in social species or as interest in external stimuli. The geckos were subjected to three testing periods: the experimental condition tested for a response to look up at a laser; a second condition was conducted without the laser; and finally, a condition without a demonstrator. The observers reliably followed the gaze of the conspecific demonstrators. Historically, reptiles have not been considered social animals; however, as social is often defined with words such as "friends" and "human society" (Merriam-Webster, n.d.) it is possible too much of an anthropocentric viewpoint is applied where there is no ability to infer such internal processes. Furthermore, recent research has shown that reptiles demonstrate a wide range of sociality and are capable of complex interactions (Doody, 2023). While leopard geckos are a solitary species that rarely interact outside of mating season, these animals are often housed together peacefully in captivity and may serve as an example of the intricate sociality often dismissed in reptiles which has been presumed to be found only in mammals and birds.

Additionally, successful target training through positive reinforcement has been demonstrated in Cuban crocodiles (*Crocodylus rhombifer*), false water cobras (*Hydrodynastes gigas*), and Aldabra tortoises, animals which are notoriously large and difficult to handle safely in captivity. Kuppert (2013) described the use of positive reinforcement to train the Cuban crocodiles at the National Zoo; these targeting behaviors allowed for the animal to be positioned in a safe and stress-free manner for veterinary procedures, including blood draws without the use of sedation. False water cobras were trained to follow a target to facilitate shifting the animal from their primary enclosure into a shift container (Williams et al., 2022). The authors found significant decreases in stress behaviors for individuals who had no previous training and, while progress was slower, training was still possible with individuals who had previous aversive experiences with training. Weiss and Wilson (2003) concluded positive reinforcement training of Aldabra tortoises *(Geochelone gigantea*) for venipuncture allowed samples to be quickly and easily collected while strengthening the relationship between animals and caretaking staff. However, no research could be found describing techniques to systematically reduce agonistic or other behavioral problems in reptiles.

Negative Reinforcement Training of Green Iguanas

Despite the frequency of them living in United States (US) homes, there are no studies on training captive green iguanas. Given the lack of training and unnatural housing conditions, green iguanas often become stressed and display agonistic behaviors (Lillywhite, 2023). Agonistic behaviors displayed by captive iguanas are potentially maintained by negative reinforcement and it would be worthwhile to investigate the use of negative reinforcement to shape alternative, non-agonistic behaviors. This type of differential reinforcement could promote non-agonistic behavior while placing aggressive or other agonistic behaviors on extinction by eliminating the motivation to offer such behavior. Also, in keeping the aversive stimulus at the lowest amount possible, the arousal level of the learner should not pass the reactivity threshold permitting acclimation to the aversive stimulus and potentially allowing for the aversive stimulus to be viewed as benign (Stewart, 2016).

This research sought to expand upon reptile training literature to determine whether negative reinforcement can decrease agonistic behaviors in a captive green iguana. This included adapting

Rentfro's (2012) work through a series of training steps from approaching a captive green iguana's enclosure through opening the enclosure door and extending a hand towards the iguana and removing the trainer contingent on non-agonistic behaviors emitted by the iguana.

Methods

Subject

The subject of this research, Gus, was a 4-year-old, male green iguana (*Iguana iguana*). Gus was adopted from a rescue group three years prior; no history was provided with his adoption. Whenever caretakers entered the room in which Gus lived, he became alert and progressed through the list of agonistic behaviors in Table 2 as the caretaker came closer to him. If Gus needed to be removed from his enclosure for cleaning or veterinary care, he fled and needed to be chased and cornered. Once in hand, he continued attempting to escape with hissing and whipping his tail while his nails shredded whatever surface they contacted, including the caretakers' skin if it was exposed.

Setting

Gus lives in a Custom Cage HR04 Reptile measuring 182.88cm tall, 243.84cm wide, and 91.44 cm deep with ten ledges of varying sizes, one perpendicular 83.82cm Y-shaped replica log supporting one angled 133.99cm straight replica log, a large water basin, and one water dish (Figure 2). The floor of the enclosure is covered in shredded coconut husk. The back is covered in a rainforest image while the remaining three sides are clear acrylic. The front of the enclosure consists of four sliding panels with two locks to secure them in place when fully closed. Attached to the opaque roof are four black dome fixtures containing two 150W heat bulbs and two 150W halogen bulbs with two 60.96cm full spectrum combination bulbs. A v5.0 Ultimate Misting System supplies a minimum of 65% humidity throughout the enclosure via four nozzles inserted through the back panel. The temperature within the enclosure ranges from 18°C to 38°C providing multiple microhabitats. The enclosure is placed against a wall in the front room of the house allowing Gus to look out through the remaining walls into the room. Also living in the house are one male and one female human, one domestic shorthair cat, and one Great Dane, all of which have access, but varying opportunities, to enter the room containing the iguana enclosure. For the duration of the study, all other animals were moved out of sight and hearing distance to provide fewer distractions for Gus who may perceive them as added threats or predators. The experimenter, who was familiar to Gus, was kept consistent throughout the study.

Procedure

General Procedures

A changing criterion design was used to assess the effects of using negative reinforcement by way of the experimenter moving away from the iguana when he emitted non-agonistic behaviors. Sessions were conducted between 09:00 and 11:30 and at least one hour after daily feeding and husbandry. Each trial was recorded as successful or unsuccessful with Gus emitting non-agonistic or agonistic behaviors respectively (Table 2). Previous literature in green iguanas described a variety of anti-predator and defensive behaviors, including head bobbing, tail whipping, and hissing which were used to develop the list of agonistic behaviors in this study (Burghardt & Rand, 1982; Kaplan, 2000). Additionally, Gus's location within the enclosure was recorded.

Three trials were conducted across the Baseline Phase before continuing onto the Intervention Phase which was divided into 41 subphases as described in Table 3 (Kazdin, 2021). A trial was marked as successful if Gus displayed only non-agonistic behaviors for one or three seconds; the length alternated between subphases at each step of the process. The experimenter moved forward to the next subphase after at least three consecutive successful trials, basing the change on the stability of Gus's response at each subphase. By varying the number of trials at each subphase, the experimenter could better detect whether Gus's behavior was influenced by the criterion change rather than a predicted pattern or cycle (Kazdin, 2021). The number of trials in each subphase are listed in Table 4. If at any point Gus emitted an agonistic behavior, the experimenter stopped and waited for the behavior to decrease in intensity towards any non-agonistic behavior. Then they closed the enclosure door if it had been opened and turned and left the room from the direction they entered. After an unsuccessful trial, the next trial criterion was decreased to the previous subphase that had been successfully completed. However, on the fourth day of the Intervention Phase, it was apparent that this reduction was not enough to keep Gus's responses below his aversive response threshold. Thus, throughout the remainder of the Intervention Phase, after an unsuccessful trial, the following trial was decreased to the step where the experimenter was when Gus emitted the agonistic behavior during the unsuccessful trial. For example, if Gus emitted an agonistic trial during subphase 19 when the experimenter was located at step 16, the following trial was conducted at subphase 16. The terminal criterion was for Gus to emit only non-agonistic behaviors for at least 20 s while the experimenter's hand was lightly touching his body.

After each trial, the experimenter walked out of sight into a neighboring room for at least 30 seconds and recorded data (Figure 3). If Gus was eating, eliminating waste, or in the bottom half of his enclosure, the trial was delayed until Gus was no longer in one of these conditions to minimize circumstances placing the iguana vulnerable to attack from predators or other threats. A Nest Cam™, centered in the room facing the iguana enclosure, provided real time feedback to a cell phone held by the experimenter when out of sight. A Nikon D850™, on a tripod centered in the room facing the iguana enclosure, recorded all sessions. All trials and sessions were timed using the Stopwatch function on the Garmin Venu® 2 Plus.

Baseline

The Baseline Phase consisted of three trials, conducted over a three-day period, which involved the experimenter approaching the iguana, opening the enclosure, and reaching in to pet Gus. This served to determine the distance between the experimenter and Gus before he emitted agonistic behaviors. Following the procedures outlined by Rentfro (2012), if the agonistic behavior was minor, the experimenter continued moving toward Gus to assess if the behavior would escalate. At the point that a more intense agonistic behavior was emitted, the experimenter stopped, closed the enclosure door if it had been opened, and turned and left the room from the direction they entered.

Intervention

In this phase, the experimenter entered the room and walked towards the iguana enclosure. The distance between the entrance to the room and the enclosure was divided into 11 locations marked with painter's tape on the floor (Figure 2). Each location was spaced approximately 0.61 meters apart; the first location was nearest to the entrance of the room, locations 8 through 11 spanned the front of the enclosure. The trial began when the experimenter entered the room, approached the iguana enclosure to the first location, and stopped to observe Gus's behavior. After 1-3 s of observing non-agonistic behaviors from Gus (see Table 4 for a chronological progression of the trials), the experimenter turned and walked out of the room. However, upon the occurrence of any agonistic behavior, the experimenter stopped walking and left the room once the agonistic behavior decreased in intensity towards any non-agonistic behavior; these trials were marked as unsuccessful. Upon the completion of at least three consecutive trials with the experimenter stopping for 1 s, trials progressed to the experimenter stopping for 3 s before turning and leaving the room. After at least three consecutive successful trials, the experimenter moved further into the room to the next location closer to the enclosure. The criterion was met when the experimenter reached the eleventh location, the furthest distance into the room while still in front of the enclosure, with the iguana performing no agonistic behaviors for three seconds in three consecutive successful trials.

After the subphases above were successfully completed, the experimenter began moving through the subphases involved in opening the enclosure door. The first of these subphases, subphase 23 overall, began with the experimenter entering the room stopping in front of the door behind which Gus was located within the enclosure, and reaching an arm out towards the enclosure door and touching the door for one second. This was followed by a subphase of maintaining contact for three seconds. The next subphases consisted of sliding the door open enough to extend their fingers around the edge of the door and leaving the door in this position for one and then three seconds. The following subphases were slowly sliding the door open about halfway, approximately 0.3 m, for a duration of 1 and 3 s. This segment of the Intervention Phase concluded with two subphases with the enclosure door fully open and the iguana performing no agonistic behaviors for one and three seconds.

The final segment of the Intervention Phase progressed contingent on non-agonistic behaviors as described in the previous subphases as the experimenter reached out to touch the iguana. The first two subphases of this section, steps 31 and 32 overall, began with the experimenter extending an arm perpendicular to their body into the enclosure through the opened door and holding it in position for one and three seconds respectively. In the next subphases, the experimenter moved their extended arm towards the iguana without making contact, for one and three seconds, and were followed by two subphases with the experimenter touching the iguana’s body. The remaining subphases had the experimenter maintain light contact with the iguana with two fingers for progressively longer durations; contact was maintained for five, eight, 12, 15, and 20 seconds as modeled by Waite and Kodak (2022). The criterion was met when the experimenter maintained contact with the iguana for twenty seconds on three consecutive successful trials.

Results

Summary

The study consisted of 521 trials conducted over 15 days. Table 5 shows a breakdown of the trials by day. The Baseline Phase consisted of three trials conducted once per day over three days. The remaining trials made up the Intervention Phase and were conducted over 12 days. The mean number of trials was 43.2 (range 3-78). The experimenter reached subphase 34 (experimenter moves extended arm into open enclosure for 3 seconds) each day of the Baseline Phase. However, each trial was unsuccessful. The highest subphase successfully reached during the Intervention Phase was subphase 34. This was achieved three consecutive times on Day 3. Figure 4 depicts all trials conducted across Baseline and Intervention Phases. The dashed line shows the subphase goal (i.e., the subphase the experimenter was trying to achieve), and the dots indicate the subphase reached.

Gus moved freely within his enclosure throughout the study. During the Baseline Phase, he was located on the 1st Shelf, the 3rd Shelf, and the 4th Shelf (Figure 2). During the Intervention Phase, Gus was located on the 1st Shelf for seven trials (100% success rate), the 3rd Shelf for 280 trials (83% success rate), the 4th Shelf for 158 trials (98% success rate), and the Hammock for 73 trials (90% success rate).

During the Baseline Phase, the agonistic behaviors Gus displayed were alert body, hissing, tail whipping, wide eyes, and rigid dewlap (Table 7). A total of 12 agonistic behaviors were displayed across the three baseline trials. During the Intervention Phase, Gus displayed an alert body, rigid dewlap, moving away from the experimenter, and head bobbing. A total of 71 agonistic behaviors were displayed. Agonistic behaviors were displayed when Gus was located on the 3rd Shelf, 4th Shelf, and Hammock; no agonistic behaviors were displayed on the 1st Shelf.

Baseline Phase

In all three baseline trials, Gus displayed agonistic behaviors when the experimenter reached subphase 34 (experimenter moves extended arm into open enclosure for 3 seconds). For the first trial, Gus was located on the 4th Shelf, on the second trial he was located on the 3rd Shelf, and on the third trial he was located on the 1st Shelf. Figure 5 displays the cumulative number of agonistic behaviors throughout the study. Agonistic behaviors observed during the Baseline Phase included an alert body, rigid dewlap, wide eyes, hissing, and tail whipping. Of these behaviors, wide eyes, hissing, and tail whipping were never observed during the Intervention Phase (Table 7).

Intervention Phase

Intervention Phase Day 1

A total of 38 trials were conducted with a 97% success rate (Table 5). Gus was located on the 3rd Shelf for the first 23 trials and on the 4th Shelf for the final 15 trials (See Figure 2 for a visual of the locations). The highest successful subphase achieved while Gus was on the 3rd Shelf was subphase 6 (experimenter pauses for three seconds at Label 3). The highest successful subphase while Gus was on the 4th Shelf was subphase 8 (experimenter pauses for three seconds at Label 4). All trials conducted while Gus was on the 3rd Shelf were successful and 93% of trials conducted while Gus was on the 4th Shelf (Figure 6). Trial 31 was the only unsuccessful trial when Gus strolled away from the experimenter during subphase 8.

Intervention Phase Day 2

Gus completed a total of 73 trials and all were successful (i.e., no agonistic behaviors were observed). Gus was located on the 3rd Shelf for the first 21 trials and moved to the 4th Shelf for all remaining 52 trials. Training began at subphase 8. The highest successful subphase achieved while Gus was on the 3rd Shelf was subphase 13 (experimenter pauses for one second at Label 7) and subphase 26 (experimenter opens enclosure door slightly for three seconds) when he was on the 4th Shelf.

Intervention Phase Day 3

On Day 3, 40 trials were conducted with a 98% success rate (Table 5). Gus remained on the 4th shelf (Figure 2) for all trials. Training began at subphase 26. The highest successful subphase achieved was subphase 34 (experimenter moves extended arm into open enclosure for 3 seconds). The first trial was the only unsuccessful trial as Gus displayed an alert body.

Intervention Phase Day 4

A total of 47 trials were conducted with a 55% success rate. Gus was located on the Hammock for the first two trials, moved to the 3rd Shelf for the next 25 trials, and moved again to the 4th shelf for the remaining 20 trials. Training began at subphase 34. All trials when Gus was on the Hammock were unsuccessful (Figure 6). When Gus was located on the 3rd Shelf, seven trials were successful (28%) and the highest subphase achieved was subphase 22 (experimenter pauses for three seconds at Label 11). When Gus was located on the 3rd Shelf, 19 trials were successful (95%) and the highest subphase achieved was subphase 17 (experimenter pauses for one second at Label 9). While on the Hammock, Gus displayed an alert body, rigid dewlap, and he moved away from the experimenter. While on the 3rd Shelf, Gus displayed an alert body, rigid dewlap, a head bob, and he moved away from the experimenter. The one unsuccessful trial while Gus was on the 4th Shelf was due to him moving away from the experimenter. A total of 31 agonistic behaviors were displayed on Day 4.

Intervention Phase Day 5

On Day 5, a total of 62 trials were conducted with an 84% success rate. Gus remained on the 3rd shelf through all trials. Training began at subphase 17. The highest successful subphase achieved was subphase 18 (experimenter pauses for three seconds at Label 9). Two trials were marked unsuccessful due to a combined alert body and rigid dewlap display and eight trials were due to a rigid dewlap only. A total of 12 agonistic behaviors, consisting of alert body and rigid dewlap, were displayed over subphases 15-19.

Intervention Phase Day 6

Gus completed a total of 70 trials with an 87% success rate (Table 5). Gus was located on the 3rd Shelf for the first 50 trials and moved to the 4th Shelf for the remaining 20 trials. Training began at subphase 16 (experimenter pauses for three seconds at Label 8). When Gus was located on the 3^{rd} Shelf, 41 trials were successful (82%) and the highest subphase achieved was subphase 20 (experimenter pauses for three seconds at Label 10). Unsuccessful trials while Gus was on the 3^{rd} Shelf were due to an alert body, a rigid dewlap, a head bob, and moving away from the experimenter. All trials when Gus was located on the 4^{th} shelf were successful (i.e., no agonistic behaviors were observed). Gus displayed a total of nine agonistic behaviors on Day 6.

Intervention Phase Day 7

Due to the difference in Gus's responding based on the location in the enclosure, the research team decided to minimize training whenever Gus was located on the 4^{th} shelf. On Day 7 of the Intervention Phase, Gus remained on the 4^{th} shelf throughout the whole morning. Thus, only three trials were conducted, all at subphase 20, and all were successful.

Intervention Phase Day 8

Gus completed a total of 61 trials with an 89% success rate. Gus was located on the 3^{rd} Shelf for 60 trials and then moved to the 4^{th} Shelf for the final trial. Training began at subphase 20. When Gus was located on the 3^{rd} Shelf, 53 trials were successful (88%) and the highest subphase achieved was subphase 25 (experimenter opens the enclosure door slightly for one second). Trials were marked unsuccessful due to a combination of alert body and rigid dewlap. The only trial when Gus was on the 4^{th} Shelf was successful during subphase 21 (experimenter pauses for one second at Label 11). There were a total of eight agonistic behaviors displayed.

Intervention Phase Day 9

A total of 40 trials were conducted with a 93% success rate (Table 5). Gus was located on the 3^{rd} Shelf for 39 trials and moved to the 4^{th} Shelf for the final trial. Training began at subphase 21. When Gus was located on the 3^{rd} Shelf, 36 trials were successful (92%) and the highest subphase achieved was subphase 27 (experimenter opens the enclosure door halfway for one second). The only trial when Gus was on the 4th Shelf was successful during subphase 27. The trials were marked unsuccessful for Gus displaying a rigid dewlap.

Intervention Phase Day 10

Gus remained on the 4th shelf throughout the whole morning. Only three trials were conducted and were set at subphase 27. All trials were marked successful.

Intervention Phase Day 11

A total of 78 trials were conducted with a 94% success rate. Gus was located on the 1st Shelf for the first seven trials and then moved to the Hammock for the remaining 71 trials. Training began at subphase 27. When Gus was located on the 1st Shelf, all trials were successful. Due to an error during these trials, the experimenter jumped from subphase 27 to subphase 29 (experimenter opens the enclosure door fully for one second) for one trial before returning to subphase 28 (experimenter opens the enclosure door halfway for three seconds) for three more trials. However, no agonistic behaviors were observed during any of these trials. When Gus was located on the Hammock, 66 trials were successful (93%) and the highest subphase achieved was subphase 22 (experimenter pauses for three seconds at Label 11). A total of six agonistic behaviors, consisting of an alert body and rigid dewlap, were displayed.

Intervention Phase Day 12

Gus remained on the 4th shelf throughout the whole morning. Only three trials were conducted and were set at subphase 22. All trials were marked successful.

Discussion

To the best of the author's knowledge, this is the first study to demonstrate the use of negative reinforcement to promote non-agonistic behaviors in a reptile and in a non-domesticated species. Despite not reaching the terminal criterion during the allotted training period, this study provides evidence for the effectiveness of using negative reinforcement to promote non-agonistic behaviors in a captive green iguana. As seen in Table 7, the proportion of total trials and the proportion of days with agonistic behaviors displayed decreased in several categories (including alert body and rigid dewlap) from the Baseline Phase to the Intervention Phase. Furthermore, three agonistic behaviors (i.e., hissing, tail whipping, and wide eyes) displayed during the Baseline Phase were not shown during the Intervention Phase. Also of note, Gus displayed fewer types of agonistic behaviors overall during the Intervention Phase than were observed in the Baseline Phase.

Initially, Gus progressed through the subphases quickly, with only two unsuccessful trials out of 151 across the first three sessions (Figure 4). However, the next session began with the greatest number of consecutive unsuccessful trials (Table 4). Two potential reasons for Gus's sudden decrease in success rate are: 1) Gus's starting location (the Hammock) on Day 4 of the Intervention Phase and 2) the possibility of the 4th Shelf serving as a hiding location.

Day 4 of the Intervention Phase began with Gus in the Hammock location (see Figure 2). While Gus frequented this location regularly, this was the first time he was in this location during the study. The Hammock is located at the front of the enclosure next to the doors while all other locations Gus used during the study are in the back of the enclosure. The difference between the Hammock and the Shelves could be as much as 91.44 cm based on the depth of the enclosure. As the previous 151 trials were conducted when Gus was in the back of the enclosure, it is likely that Gus did not have a strong learning history at the front of the enclosure. Continuing forward from the last successful step, but with Gus in a new location, may not have been the appropriate successive approximation (Cooper et al., 2019). In doing so, the aversive stimulus may not have been at the lowest amount possible which in turn would have pushed Gus's arousal level past the reactivity threshold (Stewart, 2016). After only two trials in this location, Gus moved to the 3rd Shelf, located in the back of the enclosure, thereby increasing the distance between himself and the experimenter. While on the 3rd Shelf, despite decreasing the subphase after each unsuccessful trial, only seven of 25 trials were successful. These numerous consecutive agonistic displays further indicate that the criterion for reinforcement had been set too high (Cooper et al., 2019). After the 25 trials, Gus moved again to another location at the back of the enclosure, the 4th Shelf. This is the farthest location from the experimenter. In this new location, combined with reducing the subphase criterion, 19 out of 20 trials were successful.

The other potential reason for such a dramatic change in success rate is that Gus might have used the 4th Shelf as a hiding location. Of the first 330 trials of the Intervention Phase, 45% (n = 147) were conducted with Gus on the 4th Shelf. Of those 147 trials conducted when Gus was located on the 4th Shelf, only three (2%) were unsuccessful (Figure 4). The 4th Shelf is at the back of the enclosure and at the farthest end of the enclosure from where the experimenter entered the room (Figure 2). Based on the high success rate when Gus was on the 4th Shelf in conjunction with its proximity to where the experimenter entered the room, it is likely that this location served as a hiding place for Gus. By resting in this location, Gus could hide his head in the artificial plants hanging on the wall of the enclosure thereby partially or completely blocking his view of the experimenter. The perceived initial success rate when Gus was on the 4th shelf may not have been due to active participation from Gus. If he was in a hiding place, one intended for protection from outside aversive pressures, the experimenter could have been progressing based on a lack of response from Gus rather than any acknowledgement or acceptance. Assuming this to be a possibility, the experimenter made the decision at the conclusion of Day 6 (Trial 330) to minimize training if Gus was found on the 4th Shelf. The remainder of the study included 11 trials with Gus on the 4th Shelf (100% success rate) and 177 trials conducted with Gus in other locations (91% success rate). Prior to adjusting for locations, 147 trials were conducted with Gus on the 4th shelf (98% success rate) and 183 trials on other locations (78% success rate). After the procedural adjustment, there was an increase in success rate with Gus on the 4th Shelf, from 98% to 100%, as well as when he was in other locations, from 78% to 91% (Figure 6). Additionally, the adjustment reiterates the need to consider Gus's location within the enclosure and what the corresponding next successful approximation should be.

Prior understanding of Gus's uses of the locations within the enclosure could have shortened the length of this study, however it has shed some light on other vital aspects of training and captive animal care. As noted in the introduction, green iguanas are often provided inadequate captive environments. It is generally recommended that hide boxes should be provided (Kaplan, 2000) and Gus's tendency for spending so much time in the furthest location highlights the need for some form of escape or refuge. This is further emphasized in the hierarchy of behavior change, a set of procedures designed to provide a least intrusive, effective method for behavior modification that allows the learner some control over their own circumstances (Friedman et al., 2021). Although the hierarchy places positive reinforcement above all other behavior modification approaches, it is not at the top. To provide a least intrusive intervention, the hierarchy places the medical needs of the animal at the top followed by antecedent arrangements and then positive reinforcement. Antecedents are "an environmental condition or stimulus change existing or occurring prior to a behavior of interest" (Cooper et al., 2019) and careful consideration of these stimuli and conditions, or antecedent arrangements, can provide preventative behavioral management strategies (for example, in this case minimizing training whenever Gus is in his hiding location). Indeed, as green iguanas regularly seek out dark, tight-fitting spaces when entering their shedding period (Kaplan, 2000), careful monitoring of such behaviors can provide insight as to when training times are ideal. Additionally, Gus's freedom to leave the training session by moving into his hiding location provides him with some choice and control over his own circumstances. These opportunities to control his exposure to the stressor can build resiliency for future situations in which he may not have the ability to do so, such as at the veterinarian's office (Stewart, 2016).

A closer inspection of the agonistic behaviors displayed by Gus offers additional insight into the progression made throughout this study. During the Baseline Phase, Gus displayed between three and five different agonistic behaviors per trial, some of which (i.e., hissing, tail whipping, wide eyes) were never repeated in the Intervention Phase (Table 7). Additionally, during the Intervention Phase, Gus displayed four different types of agonistic behaviors in two sessions while all other sessions showed fewer types. This is likely due to procedural differences between the two phases. During the Baseline Phase, following the procedures outlined by Rentfro (2012), the experimenter kept going past minor agonistic behaviors to see if the behavior would escalate. However, during the Intervention Phase, progress stopped at the first sign of agonistic behavior. Specifically, during the Baseline Phase the experimenter reached Subphase 34 (experimenter moves extended arm towards iguana for 3 s); however during the Intervention Phase, 94% (n= 489) of the trials were conducted with the experimenter entirely on the outside of the enclosure (see Table 4 and Figure 4). Therefore, as the Intervention Phase progressed, the experimenter would be in closer proximity to the green iguana more frequently. This may indicate that certain agonistic behaviors are more effective for avoiding aversive stimuli depending on the distance to the green iguana; for example, displaying an alert body and rigid dewlap may be helpful at greater distances while hissing and tail whipping are reserved for close encounters. Other possibilities include using particular agonistic behaviors (e.g. hissing and tail whipping) for more imminent threats or when other previously displayed agonistic behaviors (e.g. rigid dewlap) are ignored by the intended recipient.

A better understanding of such agonistic displays should provide caregivers with more knowledge to accurately recognize normal versus abnormal behaviors, in turn leading to better welfare for captive green iguanas (Broom, 2021). Unfortunately, little data exists on reptilian behavior to provide such expertise (Warwick et al., 1995). The training team in this study made every effort to utilize the peer reviewed literature available to provide precise behaviors to observe, but it is possible that some behaviors were misinterpreted. As the goal was to keep Gus at the lowest level of arousal, the experimenter stopped at the first sign of any agonistic behavior. However, such small movements could have been the starting point for many different behaviors. Examples could include the experimenter stopping for a perceived head bobbing movement when the iguana was merely shaking a droplet of water off his face; or stopping as the iguana moves away when he was just adjusting into a more relaxed state; or stopping for a perceived rigid dewlap when the iguana was preparing for better thermoregulation. Distel and Veazey (1982) provide the most in-depth description of wild green iguana behavior, but they include a caveat, "[m]any behavioral elements may take part in functionally different sequences." Furthermore, several behaviors displayed by green iguanas as defensive behaviors are comparable to those employed for thermoregulation. As heliotherms, basking in warm air rather than resting on warm surfaces, green iguanas compress their bodies and expand their dewlaps for maximum heat absorption (Kaplan, 2000). Consequently, training may have been hindered by a misinterpretation of Gus's behavior.

One consistent theme across previously successful uses of negative reinforcement training was that of the effectiveness in a shorter time frame than traditional methods (Snider, 2007; Katz & Rosales-Ruiz, 2022; Rentfro, 2012). In these studies, domestic cats and dogs were able to complete the procedure in under 2 hr. As Gus did not reach the terminal criterion, a direct comparison of the length of training is not appropriate; however, the progress made in the 13 hr across 12 days of Intervention Phase training far exceeds any progress made by Gus's caregivers in this same setting in the previous three years. Additionally, if Gus's use of the 4th Shelf as a hiding location had been correctly interpreted from the start, the total training time would have been further reduced. Another possibility for the longer timeframe in this study could include the specific lifestyle of an herbivorous ectothermic animal accustomed to spending large amounts of time basking and expending less time hunting for food. Such a lifestyle indicates a greater need to conserve energy and, as such, training during basking time may be inefficient. While a similar procedure was shown to be effective in domestic sheep, another herbivorous animal, the time frame was not mentioned for comparison (Fernandez, 2020). Furthermore, this is the first time a negative reinforcement procedure has been used in a non-domesticated species. As domesticated species are adapted to living with humans (Broom, 2021), it may be easier and faster to train them to overcome their fear of humans. However, this study does show promise of being more efficient than the suggested “nine months or so” that would be needed to promote non-agonistic behavior in captive green iguanas based on current available care manuals (Kaplan, 2000). Moreover, an efficient method for reducing fear of humans could lead to positive reinforcement training resulting in improved welfare and more positive human-animal relationships (Hosey & Melfi, 2012). Research working with negative reinforcement to promote non-agonistic behaviors has often extended into positive reinforcement to maintain such behaviors including petting and brushing cats (Rentfro, 2012), petting dogs (Katz & Rosales-Ruiz, 2022), and providing treats to sheep (Fernandez, 2020). Such interactions support more contact and familiarity to the animals while providing the caretaker more opportunities for early diagnosis of medical concerns. This, in turn, allows for less stressful veterinary treatment and further developing positive human-animal interactions and even bonds (Hosey & Melfi, 2012).

Additionally, Gus’s owners have reported several positive interactions with him up to two months after the study concluded. Prior to this study, the male caretaker reported Gus displaying an alert body with rigid dewlap and wide eyes any time he entered the room housing Gus’s enclosure. After the study, the caretaker occasionally reported a rigid dewlap observed from Gus; however, he has also been able to extend his hand into Gus’s enclosure with no agonistic behavioral displays from Gus on multiple occasions. Furthermore, the female caretaker has successfully reached into Gus’s enclosure and placed fruit next to Gus, pet Gus briefly, and even picked Gus up and carried him around the room. Such interactions indicate a long-term retention of the progress made during training.

Limitations and Future Research

The limitations of this study largely stem from having only one subject with a limited number of days for implementation of the study. Smith and Blumstein (2013) provide an overview of the various anthropogenic factors affecting personality and genetic diversity of wild and captive species. They cite evidence indicating wild species might adapt their behavior in relation to human presence (moose, *Alces alces*; Berger, 2007) which, in some cases, may allow individuals to habituate more readily. Additionally, a traumatic capture of wild-caught reptiles is likely to induce chronic stress whereas a non-aggressive capture is more likely to lead to adaptability (Lillywhite, 2023). Likewise, unintentional side effects of captive breeding may result in an individual's ability to better cope with captive environments (Smith & Blumstein, 2013). Furthermore, while wild-caught green iguanas tend to be more reactive to human presence than captive-reared individuals, their defensive displays are commonly confused with signs of stress adding to the difficulty of understanding how these reptiles respond to humans (Burghardt & Layne-Colon, 2023). As there is no available history on Gus, there is no way to be sure whether he was bred in captivity or wild-caught or whether, and to what extent, anthropogenic factors have shaped his behavioral and genetic diversity. Additional research is needed in a variety of wild- and captive-reared species to determine if these factors limit an individual's ability to succeed with this type of negative reinforcement training.

Moreover, while green iguanas are often described as lounging about basking in the sun (Burghardt & Rand, 1982), during the breeding season their behavior often changes quite dramatically. Both female and male captive green iguanas become restless, and males can become quite aggressive (Kaplan, 2000). They have been described as attempting to mate with female human caretakers or viewing male human caretakers as rivals and that the color of the human's clothing is likely to initiate such attacks (Kaplan, 2000). Frye et al. (1991) report several cases of solo-housed, captive male green iguanas (all residing in California, Florida, and Massachusetts) suddenly attacking their human caretakers, particularly females during their menstrual cycles, and suggest a cause-and-effect relationship. Other such reports have been described as well up to the point of needing a discussion of castration (Kaplan, 2000; Stahl, 2003). Unfortunately, such descriptions appear largely anecdotal with no scientific evaluation which could further promote explanatory fictions of the behavior of captive green iguanas (Cooper et al., 2019). Indeed, the current study suggests these may be explanatory fictions: the experimenter was a female, wearing a variety of colors, training a solo-housed, captive male green iguana in California. However, this is only one example, and further studies would be needed to better understand how temporal distribution, human hormonal cycles, gender (both human and iguana), and green iguana behavior may be related.

As previously mentioned, this study may have been hindered due to the lack of knowledge on green iguana behavior. As such, one potential behavior not explored in this study was the ability of green iguanas to change their coloration. Lizards are capable of rapid color changes in response to external temperatures, to facilitate social communication or camouflage, and it can vary seasonally (Warwick et al., 2023). Green iguanas have been reported to change their color based on different circumstances, specifically a darkened color when in a relaxed posture, a lighter color when excited, and darkened abdominal stripes in a defensive posture (Burghardt & Rand, 1982). This ability has been observed in juvenile green iguanas to a lesser extent than adults, but for unknown reasons (Burghardt & Rand, 1982). Green iguanas have also been known to change their coloration to darker shades when being handled by unknown humans (Kaplan, 2000). Diet has also been reported to influence longer term color changes (Kaplan, 1991). Also, both male and female green iguanas have been observed changing their coloration during the breeding season (Kaplan, 2000). The purpose of this study was to determine whether negative reinforcement could decrease agonistic behaviors in a captive green iguana towards their caretaker, so the training team chose to assess those behaviors which could be most easily detected by the typical caretaker. As it is likely that most reptile keepers have limited access to and understanding of the scientifically evaluated data (Warwick et al., 2023) on this behavior, the focus was shifted to more readily detectable behaviors. Thus, future studies could be modified to include color change to provide further insight into the role this behavior plays in the human-animal relationship.

Conclusion

Overall, this study demonstrated the use of negative reinforcement to promote non-agonistic behaviors while decreasing agonistic behaviors in a captive green iguana. Fewer types of agonistic behaviors were displayed during the Intervention Phase than were displayed during the Baseline Phase. The proportion of trials with agonistic displays also decreased in the Intervention Phase. Differences were noted depending on Gus's location within the enclosure including between locations at the front of the enclosure versus the back and the use of one specific location as a potential hiding spot. These differences indicate a need for modifications according to the animal's use of the space to facilitate efficient and effective training. This case also indicates long term retention as the caretakers have reported numerous positive interactions with the green iguana several months after training concluded.

Not only did this study provide further insight into the training abilities of green iguanas, but it also added to the greater reptilian and negative reinforcement scientific literature communities. Importantly, this study presents another example of utilizing a naturally occurring unavoidable stimulus to promote behavior change in a least intrusive manner. This procedure delivers opportunity for use in a variety of other captive species given that species-specific modifications are made.

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Table 1

Definitions of behaviors observed from a captive green iguana as adapted from Distel & Veazey (1982)

Behavior	Description
Body Position	
Laying down	Body and tail fully in contact with the ground, knees bent and feet back; the tail may hang loosely off a ledge; the head may or may not be raised, eyes closed or open, but no part of the eye above the pupil exposed
Raised	Body partially or fully raised off the ground; legs may be bent or fully extended (as shown in Figure 1)
Alert	Body and tail raised off ground; body laterally compressed, leaning at an angle away from 90°
Dewlap Position	
Retracted	Dewlap tucked up tightly under chin
Loosely extended	Dewlap extended below chin, hanging loosely, and swaying when in motion (as shown in Figure 1)
Rigid extended	Dewlap extended below chin with leading edge slanting forward
Eyes	
Closed	Eyelids closed showing no part of the eye; may be one or both eyes
Soft	Eyelids parted showing the eye, but muscles are relaxed with the upper portion of the eye remaining hidden (as shown in Figure 1)
Wide	Eyelids tense, eye fully exposed
Head	
No bob	Head may be lowered or raised or moving for a better viewing angle, but no repetitive movements seen
Head bob	Dewlap may be partially or fully extended; head raised and alternated in an up and down motion in quick succession
Shudder bob	Dewlap fully extended; head raised and rapidly alternated from side to side intermixed with up and down motions
Mouth	
Closed	Mouth fully closed with upper and lower jaw in full contact (as shown in Figure 1)
Tongue-flicking	Tongue extends past open mouth; may occur singularly or repeatedly
Hiss	Forced air from the body through the partially opened mouth
Wide open	Mouth wide open exposing arched tongue
Tail Position	
Lowered	Tail in full contact with the ground or hanging loosely off the edge (as shown in Figure 1)
Twitching	Distal half of tail is moved slowly from side to side; may or may not be raised
Whipping	Tail raised and rigid moved rapidly from one side of body to the other
Locomotion	
Stroll	Body raised minimally off the ground, frequent halts to flick tongue; slow pace
Walk	Body fully raised; tail may also be raised; no halting; brisk pace
Run	Body fully raised; rapid pace; tail waving back and forth

Table 2

Non-agonistic and agonistic behaviors of a captive green iguana as adapted from descriptions by Kaplan (2000)

Non-Agonistic Behaviors	Agonistic Behaviors
Body laying down or raised	Body alert
Dewlap retracted or loosely extended	Dewlap rigidly extended
Eyes closed or soft	Eyes wide
No head bobbing	Head bob or shudder bob
Mouth closed	Hissing
Tongue-flicking	Mouth wide open
Tail lowered	Tail twitching or whipping
Stroll or walk towards human	Stroll/walk away from human or any running

Table 3

List of subphases within the Intervention Phase

Subphase	Brief Description
Approaching Enclosure Segment	
1-2	Experimenter pauses for 1 and 3 s at label 1
3-4	Experimenter pauses for 1 and 3 s at label 2
5-6	Experimenter pauses for 1 and 3 s at label 3
7-8	Experimenter pauses for 1 and 3 s at label 4
9-10	Experimenter pauses for 1 and 3 s at label 5
11-12	Experimenter pauses for 1 and 3 s at label 6
13-14	Experimenter pauses for 1 and 3 s at label 7
15-16	Experimenter pauses for 1 and 3 s at label 8
17-18	Experimenter pauses for 1 and 3 s at label 9
19-20	Experimenter pauses for 1 and 3 s at label 10
21-22	Experimenter pauses for 1 and 3 s at label 11
Opening Enclosure Door Segment	
23-24	Experimenter extends arm to touch enclosure door for 1 and 3 s
25-26	Experimenter opens enclosure door slightly for 1 and 3 s
27-28	Experimenter opens enclosure door halfway for 1 and 3 s
29-30	Experimenter opens enclosure door fully for 1 and 3 s
Touching Iguana Segment	
31-32	Experimenter extends their arm into open enclosure for 1 and 3 s
33-34	Experimenter moves extended arm towards iguana for 1 and 3 s
35-36	Experimenter touches the iguana’s body for 1 and 3 s
37-41	Experimenter maintains contact with the iguana for 5, 8, 12, 15, and 20 s

Table 4

List of trials conducted chronologically within each subphase with successful trials followed by unsuccessful trials (successful/unsuccessful trials)

Subphase	Trials Conducted (success/fail)
Baseline	0/3
1	3/0
2	4/0
3	5/0
4	3/0
5	4/0
6	6/0
7	4/0
8	1/1
7	4/0
8	5/0
9	4/0
10	3/0
11	4/0
12	5/0
13	3/0
14	4/0
15	5/0
16	3/0
17	4/0
18	5/0
19	3/0
20	4/0
21	5/0
22	5/0
23	3/0
24	4/0
25	4/0
26	3/1
25	3/0
26	4/0
27	5/0
28	3/0
29	4/0
30	5/0
31	3/0
32	4/0
33	5/0
34	3/1
33	0/1
32	0/1
31	0/1
30	0/1
Subphase (cont'd)	**Trials Conducted (cont'd)**
29	0/1
28	0/1
27	0/1
26	0/1
25	0/1
24	0/1
23	0/1
22	3/0
23	0/1
22	0/1
21	0/1
20	0/1
19	0/1
18	1/1
17	0/1
16	3/1
15	4/0
16	3/1
15	5/0
16	3/0
17	4/1
16	4/0
17	5/0
18	1/1
17	0/1
16	5/0
17	0/1
16	2/1
15	0/1
14	4/0
15	5/0
16	4/0
17	3/0
18	4/0
19	0/1
18	3/0
19	0/1
18	1/1
16	3/0
17	4/0
18	1/1
16	5/0
17	3/0
Subphase (cont'd)	**Trials Conducted (cont'd)**
18	4/0
19	0/1
18	3/0
19	2/1
18	4/0
19	4/0
20	1/1
19	1/1
18	3/0
19	0/1
18	4/0
19	3/1
18	0/1
16	1/1
15	3/0
16	3/1
15	3/0
16	4/0
17	3/0
18	4/0
19	3/0
20	6/1
19	1/1
18	3/0
19	4/0
20	5/0
21	3/0
22	4/0
23	5/0
24	4/0
25	0/1
24	2/1
23	1/1
22	3/0
23	3/0
24	5/0
25	1/1
24	0/1
19	3/0
20	4/0
21	3/1
20	0/1
19	0/1
Subphase (cont'd)	**Trials Conducted (cont'd)**
18	3/0
19	4/0
20	3/0
21	4/0
22	3/0
23	4/0
24	5/0
25	3/0
26	4/0
27	10/0
29	1/0
28	3/1
11	3/0
12	4/0
13	4/0
14	3/0
15	3/0
16	4/0
17	3/0
18	4/0
19	0/1
18	3/0
19	1/1
18	4/0
19	3/1
17	4/0
18	3/0
19	2/1
18	4/0
19	4/0
20	3/0
21	4/0
22	6/0

Table 5

Summary of trials conducted across phases

	Total Trials	No. of Unsuccessful Trials	Success Rate (%)	No. of Agonistic Behaviors	Highest Successful Subphase
Baseline Phase	**3**	**3**	**0**	**12**	**--**
Day 1	1	1	0	3	--
Day 2	1	1	0	5	--
Day 3	1	1	0	4	--
Intervention	**518**	**57**	**89**	**71**	**34**
Day 1	38	1	97	1	8
Day 2	73	0	100	0	26
Day 3	40	1	98	1	34
Day 4	47	21	55	31	22
Day 5	62	10	84	12	18
Day 6	70	9	87	9	20
Day 7	3	0	100	0	20
Day 8	61	7	89	8	25
Day 9	40	3	93	3	27
Day 10	3	0	100	0	27
Day 11	78	5	94	6	29
Day 12	3	0	100	0	22

Table 6

Summary of trials conducted across locations

	Total Trials	No. of Unsuccessful Trials	Success Rate (%)	No. of Agonistic Behaviors	Highest Successful Subphase
Baseline Phase	**3**	**3**	**0**	**12**	**--**
1st Shelf	1	1	0	4	--
3rd Shelf	1	1	0	5	--
Hammock	0	--	--	--	--
4th Shelf	1	1	0	3	--
Intervention	**518**	**57**	**89**	**71**	**34**
1st Shelf	7	0	100	0	28
3rd Shelf	280	47	83	56	27
Hammock	73	7	90	12	22
4th Shelf	158	3	98	3	34

Table 7

Summary of agonistic behaviors displayed by Gus across phases

	Proportion of Total Trials	Proportion of Days	Locations Displayed
Baseline Phase			
Alert Body	1 (3/3)	1 (3/3)	1st Shelf, 3rd Shelf, 4th Shelf
Hissing	1 (3/3)	1 (3/3)	1st Shelf, 3rd Shelf, 4th Shelf
Tail Whip	0.66 (2/3)	0.66 (2/3)	3rd Shelf, 4th Shelf
Wide Eyes	0.66 (2/3)	0.66 (2/3)	1st Shelf, 3rd Shelf
Rigid Dewlap	0.66 (2/3)	0.66 (2/3)	1st Shelf, 3rd Shelf
Move Away	0 (0/3)	0 (0/3)	--
Head Bob	0 (0/3)	0 (0/3)	--
Intervention Phase			
Alert Body	0.03 (16/518)	0.5 (6/12)	3rd Shelf, Hammock, 4th Shelf
Hissing	0 (0/518)	0 (0/12)	--
Tail Whip	0 (0/518)	0 (0/12)	--
Wide Eyes	0 (0/518)	0 (0/12)	--
Rigid Dewlap	0.09 (45/518)	0.5 (6/12)	3rd Shelf, Hammock
Move Away	0.01 (7/518)	0.25 (3/12)	3rd Shelf, Hammock, 4th Shelf
Head Bob	0.003 (2/518)	0. 17 (2/12)	3rd Shelf

Figure 1

Anatomy of a green iguana

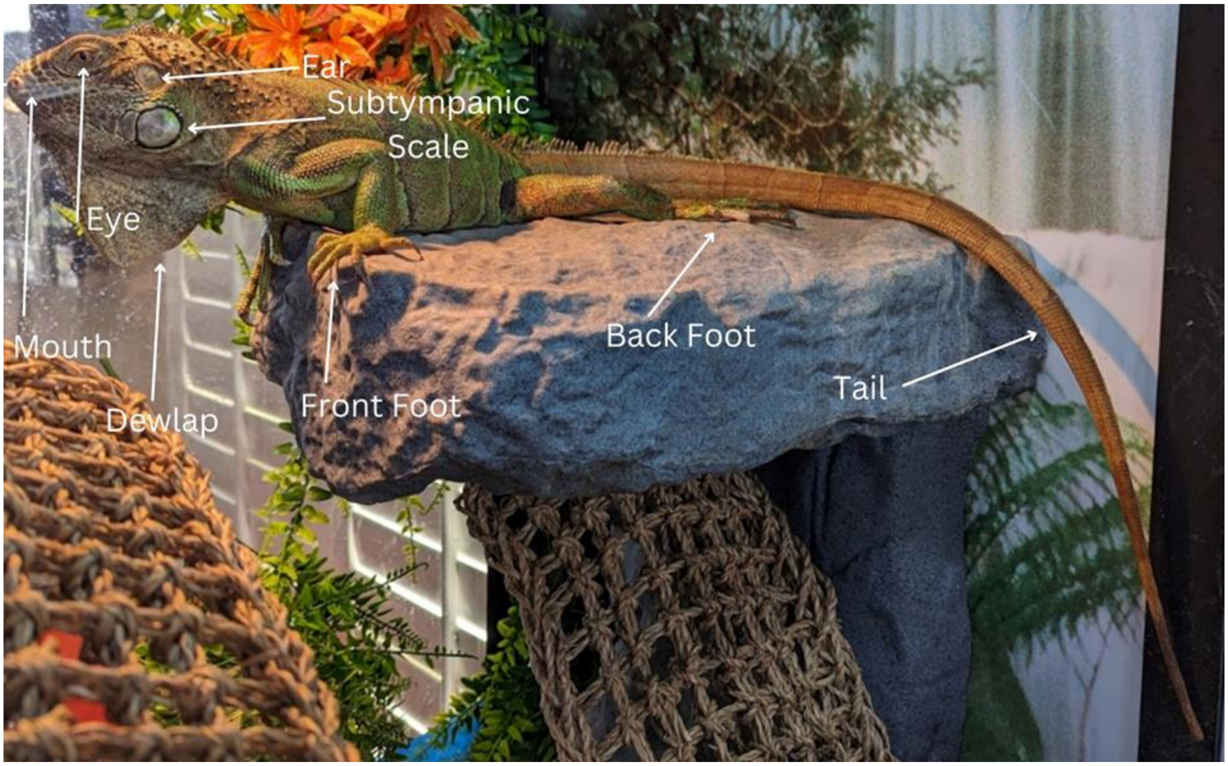

Figure 2

Iguana enclosure with labeled locations and steps in Approaching Enclosure Segment

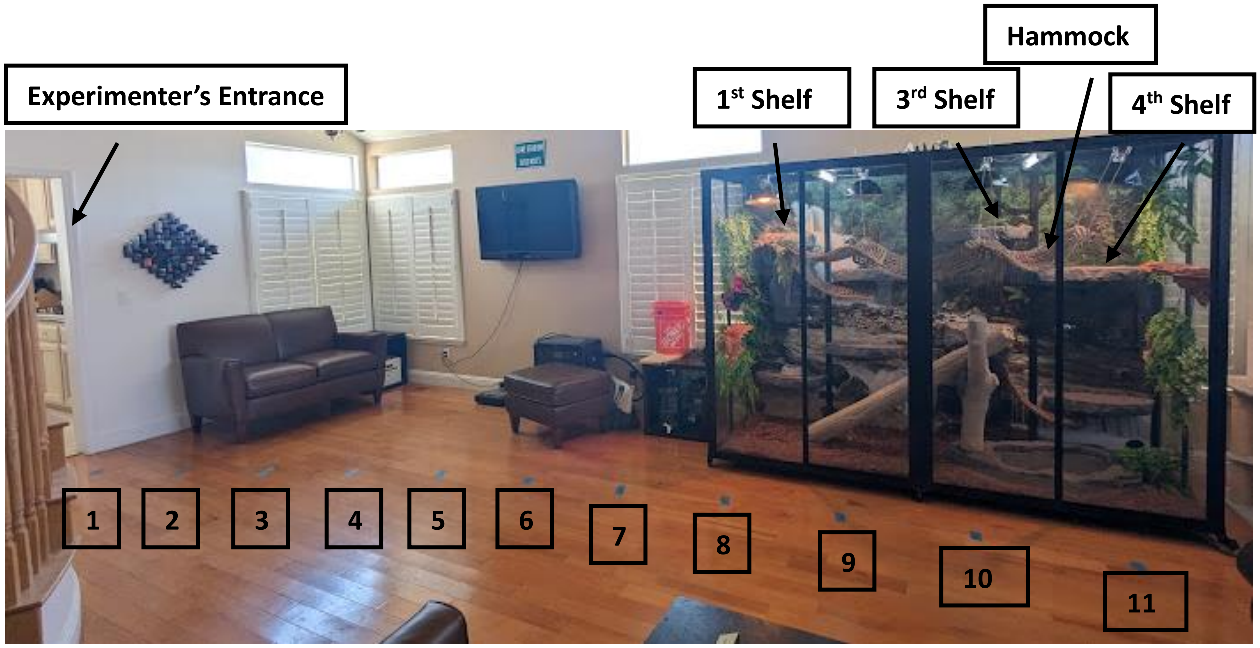

Figure 3

Data collection form

Date:				Time:		
Trial	**Time**	**Successful (Y/N)**	**Non-Agonistic Behaviors**	**Agonistic behaviors**	**Iguana's Location within Enclosure**	**Additional Notes**
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
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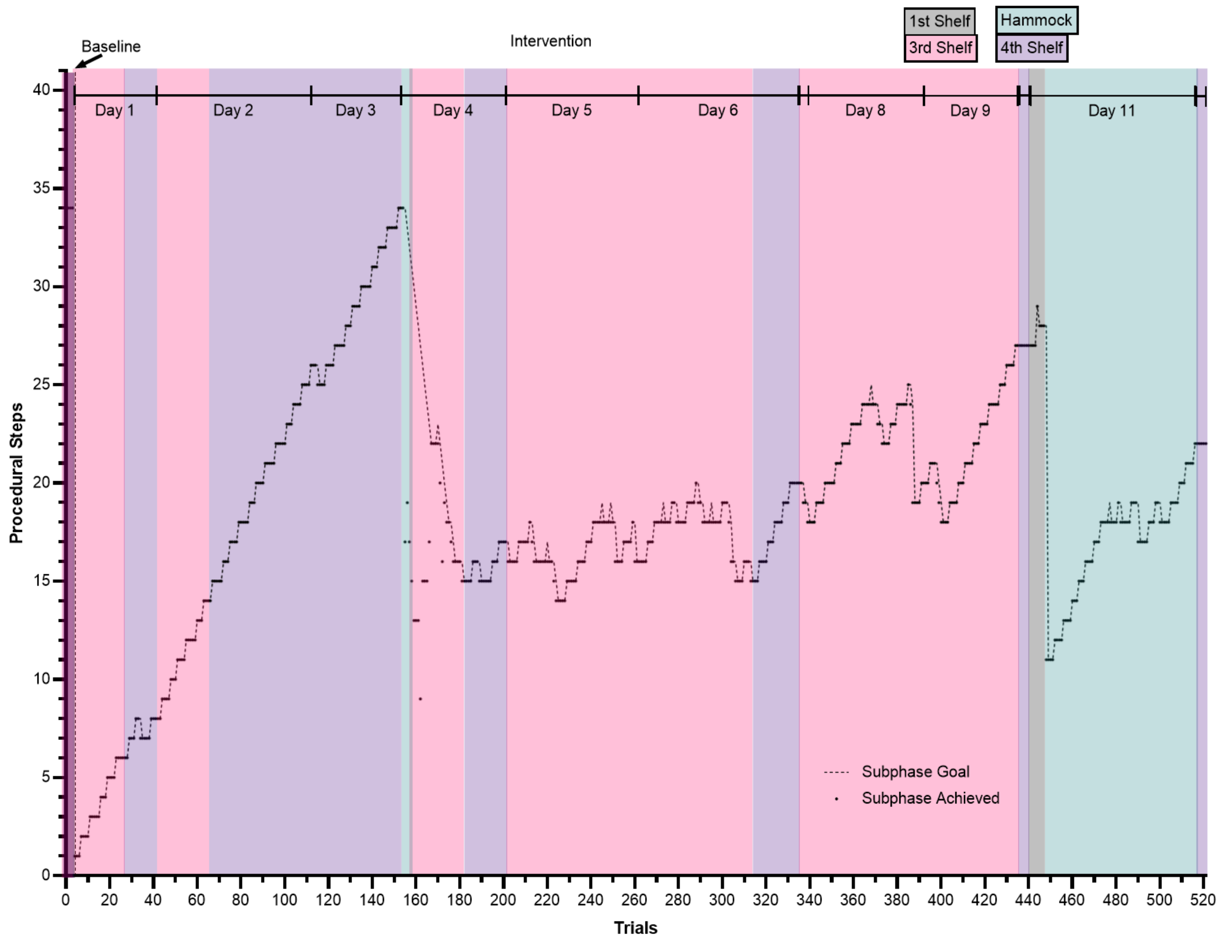
29						
30						

Figure 4

Trials conducted across subphases



Note. The dashed line represents the subphase the experimenter was trying to achieve, the solid dots indicate the subphase reached when trial was terminated, and the shading indicates Gus's location within his enclosure. The gray shading represents trials with Gus on the 1st Shelf; pink shading represents trials with Gus on the 3rd Shelf; blue shading represents trials with Gus on the Hammock; purple shading represents trials with Gus on the 4th Shelf.

Figure 5

Cumulative agonistic behaviors observed across all trials

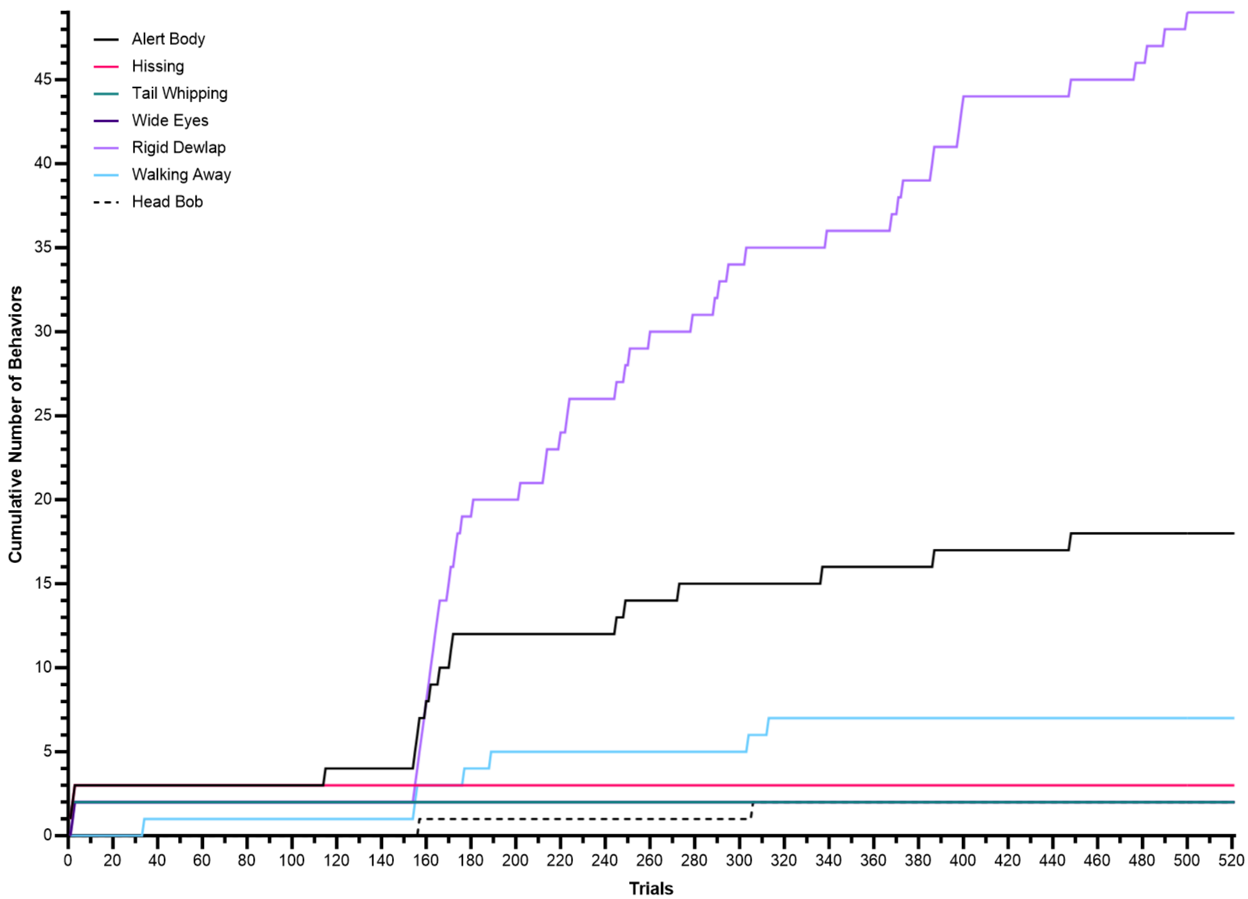


Figure 6

Proportion of successful trials across sessions and locations within the enclosure