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DO COTTONMOUTHS (*AGKISTRODON PISCIVORUS*) HABITUATE TO HUMAN CONFRONTATIONS?

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ABSTRACT - The defensive behavior of snakes towards humans has been well documented. Ironically, many of these early studies focused on harmless colubrid species. Knowledge of venomous snake defensive behavior is limited, and further research is necessary to understand how pitvipers react to human confrontations. I performed laboratory tests daily over a period of five days to investigate whether cottonmouths would habituate to handling. Eleven days after the last habituation test, snakes were tested again to see if cottonmouths show a recovery response. Cottonmouths exhibited a significant change in defensive behavior between Day 1 and Day 5 of the experiment. However, they did not significantly revert to their original behavior 11 days later.

INTRODUCTION

The defensive behavior of reptiles has received increasing attention from biologists during the last decade. Snakes are favorite subjects partly because they display the most elaborate antipredator mechanisms among reptiles (Greene 1988). On the other hand, the learning capabilities of snakes and other reptiles have been highly overlooked, probably because scientists have long questioned their intelligence.

Many factors have been found to influence a snake's defensive behavior: attack severity (Arnold and Bennett 1984), environmental and body temperatures (Goode and Duvall 1989, Keogh and DeSerto 1994, Rowe and Owings 1990, Whitaker et al. 2000), sex (Scudder and Burghardt 1983), reproductive condition (Graves 1989), and body size (Hailey and Davies 1986, Whitaker and Shine 1999). Of the learning studies that have been conducted, most have used emydine turtles, and snakes have rarely been used in habituation studies (Burghardt 1977). Wyers et al. (1973) defined habituation as a "stimulus specific response decrement resulting from repeated or constant exposure to the response eliciting stimulus." Most of the habituation experiments done on ophidians were performed in the late 1960s–1970s. Habituation of behavior in snakes has been found in prey-attack response (Burghardt 1966, Czaplicki 1975, Morris and Loop 1969), following introduction into a novel environment (Chiszar et al. 1976), as well as in defensive behavior (Fuenzalida et al. 1975, Herzog et al. 1989, Scudder and Burghardt 1983, Scudder and Chiszar 1977). Fuenzalida et al. (1975) found a short-term habituation (e.g., stimuli presented at less than 24-hour intervals) in Plains garter snakes

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(*Thamnophis radix* Baird & Girard, 1853*)* to repeated shadow stimuli. They concluded that snakes were not able to "remember" more than a few hours and they speculated that it could be the result of ineffective long-term memory capability. Likewise, three water snakes species (*Nerodia* Baird & Girard, 1853) showed a reduction of tongue flick rates when presented with different levels of threat in successive trials of 1-min intervals (Scudder and Burghardt 1983). Two rattlesnake species (*Crotalus viridis* Rafinesque, 1818, *Sistrurus catenatus* Rafinesque, 1818) showed a short-term habituation of defensive behavior to immobile threat stimuli but not to moving stimuli (Scudder and Chiszar 1977). Finally, Herzog et al. (1989) found a decline in the number of strikes in Mexican garter snakes (*Thamnophis melanogaster* Peters, 1864) but not in Butler's garter snakes (*Thamnophis butleri* Cope, 1889*)* when tested daily over a period of five days. They were the first to show long-term habituation to stimuli presented over at least 24-hour intervals in snakes. The former species also showed a recovery response to the threat stimulus 10–13 days later, in that snakes struck more frequently than on the last habituation test.

Many of the studies conducted on defensive behavior of snakes focused on harmless species. Due to the potential threat towards humans, a better understanding of pitviper defensive behavior is needed so that conclusions can be based on scientific analyses rather than on anecdotal observations. Cottonmouths are ideal for this study since they exhibit a large repertoire of defensive displays: they tail vibrate, release musk, and also mouth gape (open-mouthed display that exposes the interior white lining). The present study was designed to investigate whether cottonmouths (*Agkistrodon piscivorus* Lacépède, 1789) would show long-term habituation to daily handlings by humans (visual and tactile stimuli). I hypothesized that (1) cottonmouths would show a more passive defense to human disturbances over time, and that (2) there would be a significant recovery response (reversion to original behavior), if the data supported hypothesis 1.

MATERIALS AND METHODS

Subjects

Thirteen cottonmouths, eight females and five males (mean SVL = 667.7 mm, range = 630–705 mm), were collected from the wild. Ten were captured in Green swamp, Brunswick County, NC, in spring 2002. In addition, three gravid females were captured on the Savannah River Site, Aiken County, SC, during summer 2002. I tested the gravid females four months after parturition and none of them were emaciated. Each snake was individually housed in a polyethylene container (Rubbermaid™ [58 x 42 x 14-cm high]) with a water dish and bark mulch as a substrate. The snakes were housed in these containers three weeks prior to the test and were fed once during that period. The snakes were housed in an environmental chamber scheduled on a 12-hr light-dark cycle. The ambient temperature in the room was consistently kept at around 26 °C. Prior to the test, handling of the specimens was minimal and they were disturbed only for water changes (provided *ad-libitum*) and feeding. They were offered a meal six days before the experiment began to eliminate the influence of recent feeding on defensive behavior (Herzog and Bailey 1987). They were not fed after the experiment started.

Testing procedures

All tests were performed in the individual housing containers to limit pre-test and post-test handling, thus avoiding displacement effects. Since the heights of the containers were small (14 cm), each container was placed in a plastic-wall arena (82 x 52 x 34-cm high) prior to each test. In that way, specimens were less likely to escape, minimizing differential handling that could have biased the defensive behavior exhibited by the subjects. After placing the container into the arena, the lid was removed by hand. In each trial, the snake was approached and picked up at midbody with an artificial arm and lifted ca. 10-cm from the bottom of the container. The artificial arm consisted of 1-m snake tongs (Whitney tongs) customized to look like a real human arm (Gibbons and Dorcas 2002). The end of the tongs was equipped with a leather glove. The behavior of the snake was recorded from the initiation of approach until 20 seconds after the snake was grasped. Then, the snake was gently dropped in the container and the lid was replaced. Each snake was tested daily over a period of five days. All tests were performed in the environmental chamber between 10:00 am and 12:00 pm. An additional test was given 11 days after the last habituation trial (Day 16) to test whether a recovery response to human disturbances had occurred. Each test was videotaped using a digital video camera to allow the observers to review each trial. The principal investigator recorded behavioral responses during initial filming, in addition to another observer. Then, the principal investigator and the independent observer reviewed the tapes. In cases when the two observers did not record the same behavioral response on a given trial, they reviewed the trial together until agreement was achieved.

Behavioral scores

Each snake was given a score following revised protocols used by Arnold and Bennett (1984), Herzog and Burghardt (1986), Keogh and DeSerto (1994) and Schieffelin and de Queiroz (1991). The protocols used in these earlier experiments had to be adapted to the species tested and to the methodology used to fit this experiment. For each cottonmouth, a score was determined by observing the behavioral state of the snake's head and tail and whether or not the snake released musk. Five categories were assigned for the head, two for the tail, and two for release of musk (Table 1). I recorded gaping behavior when the snake exposed the white lining of its mouth. A feint strike was characterized as a head movement toward the stimulus with no contact between the fangs and the artificial arm (e.g., mouth closed). A bite was defined as any contact made between the snake's fangs and the arm. No distinction was made between an actual bite and a dry bite since no reliable methods could be used to assess whether or not venom was injected. I also monitored whether or not the snake twitched its tail against the substrate generating a weak auditory signal (tail vibrating). In addition, I recorded whether or not the snake released an odoriferous substance from its cloaca producing an unpleasant odor (musking). An overall score for each cottonmouth was obtained by adding the scores for each component with a possible range from 0 to 6. If a snake used several head displays during a trial, the most active defensive behavior (defined as the behavior with the highest score [Keogh and DeSerto 1994]) exhibited was recorded.

Statistical analysis

As the data failed to meet the assumptions of parametric tests, I used Friedman ANOVA to test the hypothesis of no habituation between Day 1 and Day 5. This test assumes that the variables under consideration were measured on an ordinal scale. I used a Cochran *Q*-test to test for a significant change in the presence or absence of attributes (e.g., striking, tail vibrating, tongue-flicking, gaping) between Day 1 and Day 5. The Alpha level was set at 0.05 and all tests were performed using the statistical software *Statistica'* (StatSoft Inc., 98 Edition).

RESULTS

Cottonmouths exhibited a significant difference in defensive behavior between Day 1 and Day 5 of the experiment (Fig. 1; Friedman test, $\chi_r^2 = 6.40$, df = 1, $P < 0.02$). Of the 13 snakes, two showed high consistency throughout the experiment: one showing active defense (highest score: 5; lowest score: 4) and the other being relatively mild-mannered (highest score: 2; lowest score: 1). The other 11 snakes showed much within-individual variability in their behavioral scores (Fig. 2).

For head displays, only the display with the highest score per snake was taken into account. Upon further investigation of the frequency of behavioral responses for each day (Fig. 3), it was apparent that the striking response was partly responsible for the difference, as

Table 1. Scoring scheme of behavioral responses of cottonmouths.

Component	Scoring scheme	Score
Head	No display	0
	Tongue-flicking	1
	Gaping	2
	Feint strike	3
	Bite	4
Tail	No display	0
	Tail vibration	1
Musk	No release	0
	Release	1

this display decreased steadily and constantly throughout the experiment (Cochran *Q*-test, $Q = 5.0$, df = 1, $P = 0.025$). On Day 1, nine snakes bit whereas only four snakes did so on Day 5. Looking at the number of strikes per snake per trial, on Day 1, one snake bit three

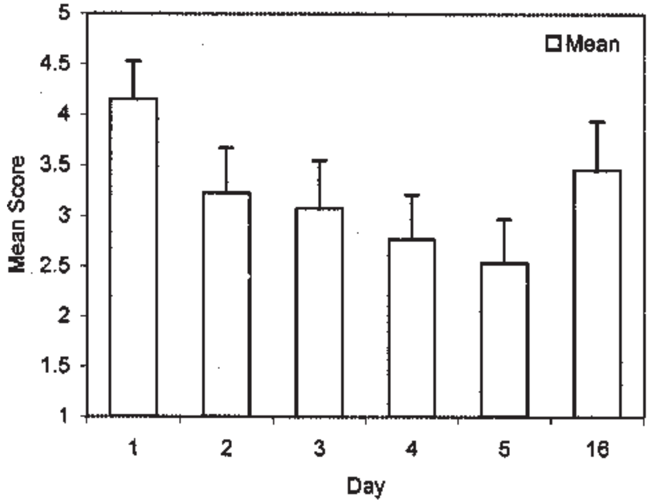

Figure 1. Mean behavioral scores of cottonmouths (n=13) per day (significant difference in defensive behavior is between Day 1 and Day 5 only [Friedman test, $\chi_r^2 = 6.40$, df = 1, $P < 0.02$]). No significant difference was found between Day 5 and Day 16 or between Day 1 and Day 16. Mean reported with 1 SE.

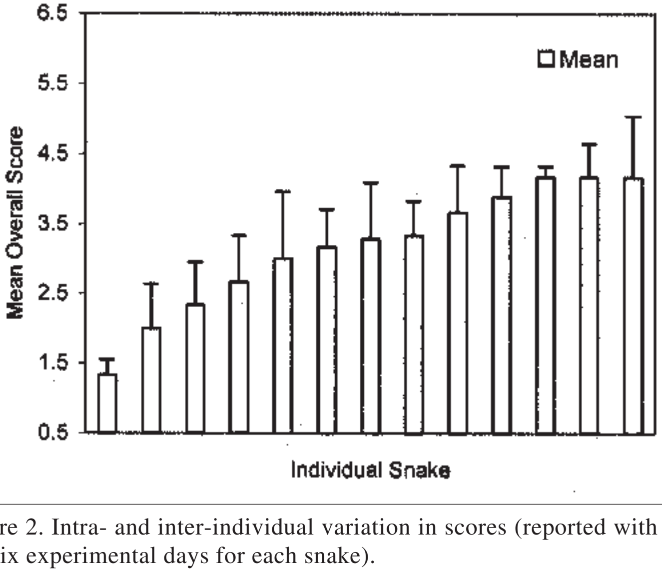

Figure 2. Intra- and inter-individual variation in scores (reported with 1 SE for the six experimental days for each snake).

times, another one bit two times, and the remaining seven only once whereas on Day 5, the four snakes only bit once. I observed a similar difference in the frequency at which snakes vibrated their tail between Day 1 and Day 5 (Cochran *Q*-test, $Q = 5.0$, df = 1, $P = 0.025$). Seven snakes vibrated their tail on Day 1 while only two did so on Day 5. On the other hand, a response substitution within head displays was observed in that the frequency of tongue-flicking behavior (as the head display with the highest score per snake) increased significantly throughout the days of the experiment (Cochran *Q*-test, $Q = 6.0$, df = 1, $P = 0.014$). On Day 1, none of the snakes only tongue-flicked while on Day 5, six of them did so. However, the frequency of gaping behavior was relatively static and showed no trend (Cochran *Q*-test, $Q = 1.0$, df = 1, $P = 0.31$).

No significant recovery response was observed when snakes were tested 11 days after the last habituation test. Snakes behaved more defensively on Day 16 than on Day 5, but the overall mean scores were not significantly different (Friedman test, $\chi_r^2 = 2.27$, df = 1, $P = 0.13$). Further, no significant difference was detected in overall mean scores between Day 1 and Day 16 (Friedman test, $\chi_r^2 = .81$, df = 1, $P = 0.36$) when eight of 13 cottonmouths (61%) bit as compared to nine (69%) on Day 1.

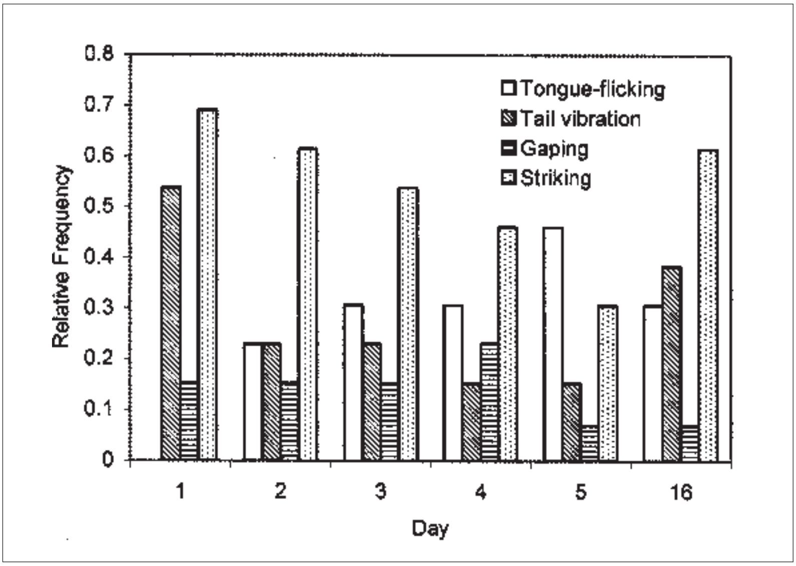

Figure 3. Frequency of four major behavioral responses per day (for head components, only the display with the highest score per snake is reported [n = 13]). Significant differences for tongue flicking (Cochran Q-test, Q = 6.0, df = 1, P = 0.014), tail vibrating (Cochran Q-test, Q = 5.0, df = 1, P = 0.025), and striking (Cochran Q-test, Q = 5.0, df = 1, P = 0.025). No significant change was found over time in the gaping behavior.

DISCUSSION

The results of this experiment show that cottonmouths (*A. piscivorus*) do habituate to handling by humans in that their behavioral responses become increasingly passive after being handled over several days. The final test (Day 16), although not significant, suggests that snakes may recover their original behavior if allowed more time between the last habituation trial and the recovery response test.

Chiszar et al. (1976) suggested that snakes might not be able to retain stimulus-specific information over a 24-hr period. From this study, it is clear that the cottonmouths habituated to daily handlings, and thus it is reasonable to say that cottonmouths have the ability to remember for more than 24 hours. Fuenzalida et al. (1975) found within-days habituation in Plains garter snakes (*T. radix*) but failed to show any between-days habituation. Herzog et al. (1989) also found that a closely related species, Butler's garter snake (*T. butleri*), did not habituate to either a non-moving or a moving human hand when tested at ca. 24 hour-intervals. However, in the same study, Mexican garter snakes (*T. melanogaster*) showed a significant decline in striking frequency between five successive days. This suggests that interspecific differences in habituation rates and/or capabilities exist, even among closely related species (i.e., congenerics). Interspecific differences in habituation capabilities might be expected as a general rule since species evolved in different ecological contexts and thus are under diverse selective pressures. However, the various methodologies and stimuli used among these studies may account for the variability observed in defensive habituation. Species have different sensory capacities and therefore, certain cues (or stimuli) may or may not elicit the same response. For instance, if testing pitviper species, one should take into consideration that pitvipers have loreal pits that enable them to detect variation in temperature (Bullock and Diecke 1956). In the present study, the artificial arm was not warmed, as would be a real human arm. If thermal cues can influence the defensive behavior of cottonmouths, the temperature of the arm could have had an impact on the results. Olfactory cues might also be important, as demonstrated for rattlesnakes during predatory strikes (Graves and Duvall 1985) and in defensive responses to ophiophagous snakes (Bogert 1941, Weldon and Burghardt 1979). Accordingly, in a study on the defensive behavior of northern Pacific rattlesnakes (*Crotalus viridis oreganus* Holbrook, 1840) to California ground squirrels (*Spermophilus beecheyi* Richardson, 1829), snakes showed no evidence of habituation when tested repeatedly with a stimulus that looked, smelled, acted, and presented the thermal profile of a ground squirrel (Rowe and Owings 1990). The common theme of all these studies is that snakes are not simple automatons in their defensive behavior, but make sophisticated judgments about their enemies and are capable of learning.

In this study, there was no need to introduce any kind of novel stimulus subsequent to each trial in order to investigate whether the decrease of behavioral scores was due to fatigue since each snake was tested at 24-hour intervals. I did not stagger the starting points of the experiment for each snake, which could potentially have resulted in a day effect (e.g., lunar cycle); however, the animals were maintained in a light- and temperature-controlled environment to minimize such effects.

The daily decrease in response score was primarily due to a steady drop in the striking rate over the five days (Fig. 3). Also, tail vibration displays showed a significant decrease between Day 1 and Day 5. No apparent trend in the frequency of gaping behavior was observed over time. Assuming the habituation demonstrated in this study is due to the ability of cottonmouths to retain information, two possible explanations exist for why they show the observed learning capability: first, the costs of striking for a venomous snake could potentially be high (e.g., risk of breaking fangs, depletion of venom supply needed for prey, energetic costs associated with venom production, etc. [Hayes et al. 2002]). Thus, it would be adaptive for a snake to modify its original behavior to another that is less costly than striking. Second, sedentary habits of cottonmouths (Tinkle 1959) may account for higher memory capacity compared to that of wide-ranging active species due to fewer encountered novel stimuli. However, exposure to novel stimuli has been shown to enhance learning abilities and brain development, in higher vertebrates at least (Nottebohm et al. 1986).

Weak evidence exists that a recovery response occurred 11 days after the last habituation test. Snakes may have higher memory capacity than suggested by some authors, and cottonmouths may be able to retain stimulus-response information for more than 11 days. Repeating this experiment and lengthening the period between the last habituation trial and the recovery response test could possibly reveal how long it takes for cottonmouths to return to their original behavior under the present context. Future studies should also consider testing other species, venomous and non-venomous, to investigate how much interspecific variation exists in defensive habituation. Also, factors such as size and reproductive condition, with differential costs and benefits, may affect the ability of a snake to habituate and thus intraspecific variation in habituation may be expected. Even though laboratory experiments can prove very useful in investigating the learning capabilities of a species, experiments in more natural settings should be performed: snakes implanted with radio-transmitters could be tracked and tested for habituation on a regular basis without being removed from their natural habitats.

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