

THE ROLE OF LEARNING IN THE EXPRESSION OF
RATTLING BEHAVIOR IN WESTERN
DIAMONDBACK RATTLESNAKES,
CROTALUS ATROX

By

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CHAPTER I

LEARNING IN RATTLESNAKES: ISSUES AND ANALYSIS

This chapter reviews research on learning in rattlesnakes. In the course of this review I briefly discuss the varieties of learning, point out necessary control groups, illuminate some of the issues to be considered when conducting research on learning in rattlesnakes, and present tables that will allow one to quickly focus on necessary research areas. I will close with a series of recommendations.

This is the first time a review has been dedicated solely to learning in rattlesnakes. There have been two earlier reviews of the reptile literature but none has focused directly on rattlesnake learning (Burghardt, 1977; Suboski, 1992). There is also a need to present a concise description of learning paradigms and discuss several issues related to the investigation of learning phenomena to an audience not familiar with traditional laboratory learning paradigms. Surprisingly, there are not many studies on rattlesnake learning.

Several factors can be cited to explain why there are so few learning studies. First, rattlesnakes are difficult to study because they are generally inactive and eat relatively infrequently. The latter presents problems because it is difficult to find suitable rewards that can be incorporated into experimental designs. Second, they are venomous and therefore can be handled only by highly trained individuals. Third, there are no commercially available apparatus such as “Skinner boxes” and “classical conditioning

chambers.” Fourth, the current “cognitive” emphasis for the study of learning suggests that rattlesnakes are inappropriate because of their less developed brains. Fifth, there is a lack of knowledge of traditional learning procedures and paradigms on the part of rattlesnake researchers. Indeed, one of the goals of this chapter is to stimulate interest in learning research and generate an understanding of appropriate methodologies.

As this volume illustrates, I believe the time is right to begin a concentrated and sustained effort to incorporate the behavior of rattlesnakes into theories of learning. Rattlesnakes have much to recommend them for such studies. Reptiles in general and rattlesnakes specifically, are noticeably missing from a phylogenetic analysis of learning (Bitterman, 1965). Bitterman (1965), for example, included learning data derived from his studies on turtles (*Chrysemys* and *Trachemys*) as “reptile-like.” My current understanding of reptile relationships (see Figure 1.1) suggests that this is a dangerous assumption, the highly specialized morphology of turtles, notwithstanding. In my view, a comparative analysis of learning cannot be complete unless rattlesnakes are considered.

Rattlesnakes are also in a unique evolutionary position in that they retain many plesiomorphies common to all snakes. This includes limblessness and a reliance on chemical information to assess the environment. However, they also possess many synapomorphies. For example, they exhibit a complex venom delivery system and a rattle. It would be of considerable theoretical and practical interest to know whether the behaviors related to these common and unique features of rattlesnakes are susceptible to experience.

In addition to the unique evolutionary position of rattlesnakes, a large amount of information is known about rattlesnakes’ natural history and maintenance in captivity

(Altimari, 1998). Such information makes them useful as laboratory subjects. Snakes are easily collected in the field or purchased from commercial suppliers. Moreover, with the proper safety precautions, laboratory technicians and students can perform experiments with relatively little risk, facilitating data collection.

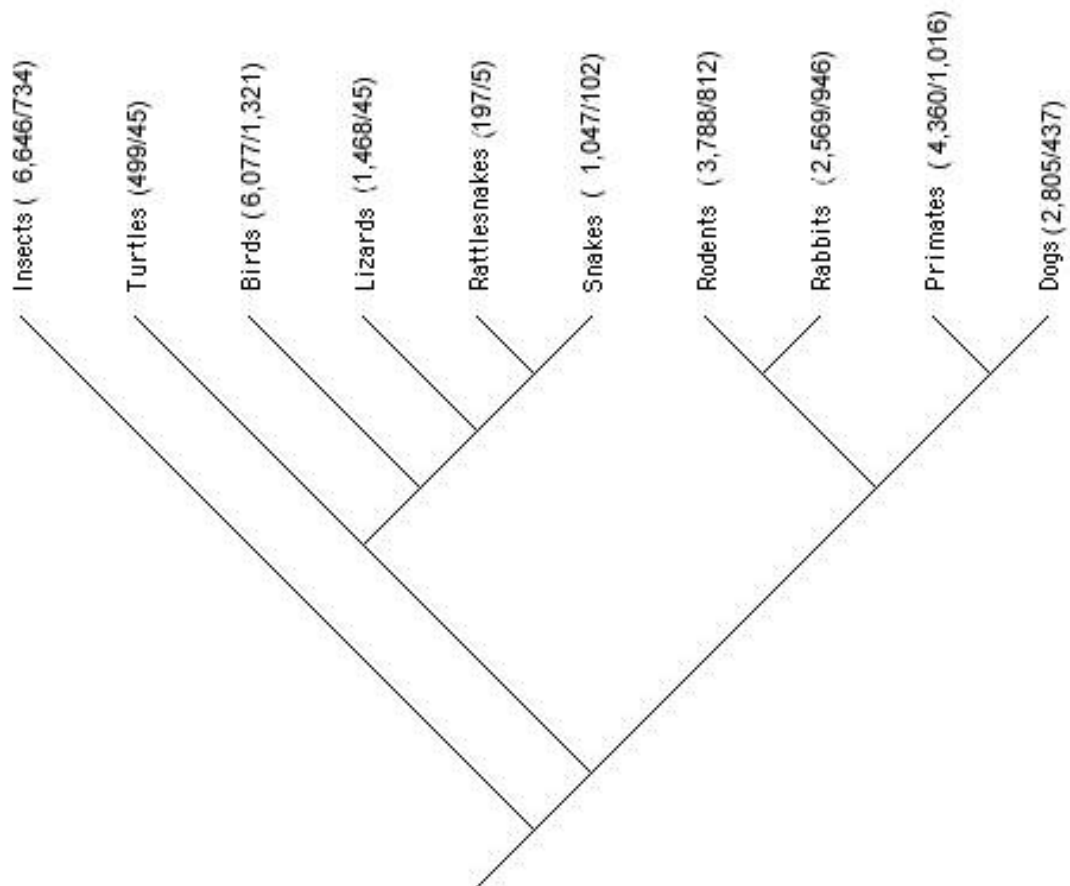


Figure 1.1. Phylogenetic relationships of animals commonly used in behavioral learning studies. Numbers in parentheses indicate the number of behavior studies and the number of learning studies. Note many of the learning studies involving snakes are psychological studies of snake phobias and not studies of learning by snakes.

Another characteristic that makes rattlesnakes important for learning research is that learning is probably involved in many ecologically relevant situations such as mate selection, predator avoidance, and foraging behavior. For example, Brown (1993, 2005) has suggested that the handling of timber rattlesnakes (*Crotalus horridus*) by human observers may be associated with specific basking sites (the so called, “spook factor”), and results in snakes avoiding those sites in subsequent years. Moreover, Clark (2004c) has shown that prior experience affects ambush site selection in timber rattlesnakes. While learning may be involved in these situations, the mechanics of learning are poorly understood. The rigorous laboratory approach I advocate in this chapter will allow us to better explain these and other phenomena.

Despite the obvious advantages of studying learning, there are surprising few formal studies. Figure 1 shows how neglected the study of rattlesnake learning is in relation to other animals traditionally used in laboratory investigations. I conducted a search on the ISI Web of Knowledge for the last 24 years (1981-2005). Each animal is represented by two categories: behavior studies and learning studies. The search was performed by querying a specific taxon and the term behavior or the term learning. I concede this search is subject to some error, but I assumed that by limiting the search to a taxon and behavior or learning most of the resulting studies would be relevant. The proportion of studies involving behavior and learning for each taxon illustrates how neglected rattlesnakes are in these areas relative to more traditional laboratory animals. Figure 1 highlights what a fertile area of research the study of rattlesnake learning is.

Types of learning

Learning is generally defined as a relatively permanent change in behavior potential as the result of experience (Zimbardo, 1992). This definition contains several important principles. First, it must be noted that learning is inferred from behavior. Learning is never observed directly. Rather, the process identified as learning is implied from observable data. Second, learning is the result of experience. This excludes changes in behavior produced as the result of development, fatigue, adaptation, circadian rhythms, or motivation. Third, temporary fluctuations are not considered learning. Rather, the change in behavior identified as learned must persist, whenever such behavior is appropriate. A fourth principle found in the definition is that more often than not, some experience with a situation is required for learning to occur. To better understand the process of learning and uncover the underlying mechanisms, psychologists have divided the mechanisms of learning into non-associative and associative categories.

Non-associative learning

Non-associative learning is a form of behavior modification involving the association of one event, as when the repeated presentation of a stimulus leads to an alteration of the frequency or speed of a response. Non-associative learning is considered the most basic of the learning processes. The participant does not learn to do anything new or better; rather the innate response to a situation or to a particular stimulus is modified. Habituation and sensitization are two types of non-associative learning that have received the most attention.

Associative learning

Associative learning is a form of behavior modification involving the association of two or more events, such as between two stimuli, or between a stimulus and a response.

Standard examples include classical, instrumental, and operant conditioning. In associative learning, the participant does learn to do something new or better.

Associative learning differs from non-associative learning by the number and kind of events that are learned and how the events are learned. Another difference between the two forms of learning is that non-associative learning is considered a more fundamental mechanism for behavior modification than is associative learning.

This can easily be seen as one moves through the animal kingdom. Habituation and sensitization are present in all animal groups, but classical, instrumental, and operant conditioning are not. In addition, the available evidence suggests that the behavioral and cellular mechanisms uncovered for non-associative learning may serve as the building blocks for the type of complex behavior characteristic of associative learning. The term associative learning is reserved for a wide variety of classical, instrumental, and operant procedures in which responses are associated with stimuli, consequences, and other responses.

Issues

Several issues must be considered before embarking on a research program to investigate learning in rattlesnakes.

Inconsistencies in definitions

Within the area of learning, there is the lack of consensus among researchers as to the definitions of many learning phenomena. I will illustrate this with examples from classical conditioning, and operant conditioning.

In contrast to definitions of habituation and sensitization, the definition of classical conditioning is not always consistent among behavioral scientists. It is important to recognize this lack of consistency when evaluating studies of classical conditioning and designing experiments. Some psychologists, for example, might stress that classical conditioning is the learning of relationships between cognitive events and that conditioning cannot be defined in terms of behavioral change. Other psychologists stress that it may be incorrect to lump together various disparate procedures under the general category of classical conditioning.

Within these extremes are definitions stressing that the neutral or conditioned stimulus (CS) must not elicit - prior to training - the response that is to be conditioned, those stressing the contingency between the CS and biologically relevant unconditioned stimulus (US), and still others stressing that classical conditioning is a procedure for creating a new reflex. On the other hand, a zoologist or herpetologist might consider classical conditioning to be the pairing of a “search image” with a sign stimulus or innate

motor program. For the physiologist, the CS might be considered solely in terms of the electrical stimulation of afferent fibers.

Classical conditioning.--In considering the problem of what is classical conditioning, Gormezano and Kehoe (1975) and Gormezano et al., (1983) describe four variations of classical conditioning based on the nature of the conditioned response (CR): (1) Conditioned Stimulus-Conditioned Response (CS-CR), (2) Conditioned Stimulus-Instrumental response (CS IR), (3) Instrumental Approach Behavior and (4) Autoshaping.

1. The Conditioned Stimulus-Conditioned Response paradigm is considered to represent the “pure” case of classical conditioning. Here the CS does not elicit the unconditioned response (UR) prior to training, and the CR emerges from the same effector system as the UR. For example, consider a hypothetical experiment in which a novel odor (e.g., peppermint) is introduced into the enclosure of a rattlesnake as a CS immediately before the enclosure is opened by the investigator (US). When the enclosure is opened, the snake vigorously rattles its tail (UR). Initially, the novel odor does not elicit a response. However, following several pairings of the odor and the opening of the enclosure, the snake will rattle vigorously when the odor is introduced (CR) and before the enclosure is opened.

2. The Conditioned Stimulus-Instrumental Response paradigm contains those experimental designs commonly known as “transfer of control: or classical-instrumental transfer” in which classical conditioning is assessed not directly but by its influence on instrumental or operant responding. Perhaps the most well-known example of this design is the conditioned suppression procedure in which a CS is paired, for example, with

electric shock (US) and the ability of the CS to suppress on-going behavior is assessed. Consider a rattlesnake that receives a novel odor (CS) paired with the presentation of a live dog (US; similar to Scudder and Chiszar, 1977) 20 times in succession. Following the pairing, the snake is moved to a new chamber and its base rate of exploratory behavior is recorded as is the number of tongue flicks/minute (Chiszar et al., 1976). The CS odor is then administered and the exploratory behavior determined. If conditioned suppression is evident, the number of tongue flicks should be less than the base rate.

3. The Instrumental Approach Design is a type of conditioning in which some approach behavior to a CS is necessary to receive the US. This procedure is illustrated by general activity to stimuli preceding food (i.e., conditioning of general activity) and instrumental runway and maze situations in which movement toward the food source is necessary. Choice of ambush sites by timber rattlesnakes (Clark, 2004c) may fit this paradigm. Woodchuck is not a typical prey item in the diet of *C. horridus*, and the test subjects used by Clark were captive-born individuals. Thus, woodchuck odor is a CS. The mouse prey can be considered the US. The UR is the ambush posture taken by the snake. On subsequent trials, the snakes took up ambush postures adjacent to the woodchuck scent trail.

4. Autoshaping is related to the CS-CR paradigm with the interesting property that the CR is not from an effector system that is related to the US. Consider the hypothetical situation in which an LED light (CS) is paired with a rodent extract (US) placed directly within its nostril. The UR would be flicking its tongue toward the light or striking the light.

It is important to note that all four categories differ in many ways: how the CR is measured; the accuracy with which the CS and US are presented in a response independent fashion; the nature of the target response; the amount of control the experimenter has over the training variables; and the degree to which the animal is restrained in the conditioning situation.

In their discussions of classical conditioning, Gormezano and Kehoe consider the CS-CR paradigm the only unambiguous case of classical conditioning. It is no accident that much of what I know about the physiology and biochemistry of the classical conditioning of vertebrates comes from the rabbit nictitating membrane preparation, which uses the CS-CR paradigm (Gormezano, 1984; Byrne, 2003).

It is worth noting that Gormezano and Kehoe do not consider alpha conditioning an example of classical conditioning. Alpha conditioning is a “conditioned stimulus” that already, prior to training, elicits a small version of the conditioned response. For example, ground born vibrations of 10 Hz might elicit a weak rattle response (say, 40 db), whereas ground born vibrations of 50 Hz might elicit a strong rattle response (say, 85 db). By pairing the 10 Hz vibrations with the 50 Hz vibrations in succession, a strong rattle response will come to be elicited by the 10 Hz vibrations if alpha conditioning occurs.

I believe that equating alpha conditioning with classical conditioning may inhibit any search for the evolutionary precursors of classical conditioning. If alpha conditioning, best characterized as the association of two unconditioned stimuli, and classical conditioning, best characterized as the association of a neutral stimulus with an unconditioned stimulus, are considered identical then certain questions become

impossible to ask. For instance, which animal taxa were the earliest to associate neutral events?

Moreover, the case has been made that alpha conditioning might actually be an example of instrumental conditioning (Razran, 1971). Let us consider a situation in which an experimenter presents a rattlesnake with a chemosensory CS which elicits, prior to its pairing with a US, a small number of tongue flicks. The US is a second chemosensory stimulus that elicits a large number of tongue flicks and over the course of CS-US pairings, the number of tongue flicks to the CS increases. Is this classical conditioning? Unfortunately, many behavior scientists would say yes. The results can just as easily be interpreted as a case of instrumental conditioning where a response to a stimulus is strengthened by a reward (having access to the second chemosensory stimulus).

Instrumental and Operant Conditioning.--Instrumental and operant behaviors are examples of associative learning in which the behavior of the participant is controlled by the consequences of behavior. Instrumental and operant behavior can be explored using a variety of apparatusa such as running wheels, runways, mazes, shuttle boxes, and lever-press situations. Instrumental and operant behaviors are generally thought to be more complex than classical conditioning. One might roughly characterize the difference by saying that classical conditioning describes how associations between stimuli are made, and instrumental and operant conditioning describe how stimuli are associated with an animal's own motor actions. Classically conditioned behavior emphasizes sensory integration, and instrumental and operant behavior, motivation: new behaviors are learned in order to obtain or avoid some stimulus. In addition, instrumental and operant

behaviors are thought to be more complex than classical conditioning because learning depends on the participants own behavior and usually requires an obviously new behavior. Despite these differences, instrumental and operant behavior share many properties with classically conditioned behavior. These include extinction, spontaneous recovery, generalization, and discrimination.

As originally conceived, operant behavior is characterized by the “goal-directed” motor manipulation of the environment (Lee, 1988). In place of the goal-directed modification of behavior that was the hallmark of the Skinnerian system, operant conditioning now generally consists of any behavior sensitive to response-reinforcer contingencies. Thus, operant conditioning is now considered to include such procedures that modifying body position, running against a taxic or kinetic preference, and learning various mazes and runways. For example, Mills (1970) utilized lateral head movements by restrained sidewinders and Mojave rattlesnakes (*C. cerastes* and *C. scutulatus*, respectively) to escape subcutaneous shock signaled by an infrared light. Individuals did learn to escape the shock by moving their heads laterally. This response is a modification of body position and is best described as an example of instrumental conditioning.

I believe that instrumental behavior is not as complex as operant behavior and that there should be a distinction between the two. It is important to note that instrumental conditioning procedures may not constitute operant behavior. Instrumental conditioning requires an organism to complete a task using an innate behavior. Navigating through a maze or running in a wheel are examples of instrumental conditioning. A major requirement of operant conditioning has been that species-typical behavior is minimized by interjecting a “novel” behavior such as a lever press, or a non-arbitrary response

brought under the control of a discriminative stimulus or cue placed between the animal and the animal's reception of some consequence. In this way, the experimenter demonstrates that the animal has learned not only how to operate some device but also "how to use it."

One might be more confident that snakes are engaging in operant behavior if it can be shown that: (1) the operant responses minimize species-typical behavior, (2) some property of the response class such as its rate, force, or interresponse time can be modified, (3) the response no longer occurs when such responses postpone the delivery of reward, and (4) the response can be brought under the control of a cue (i.e., discriminative stimulus).

A classic example of operant conditioning is the work of Kleingenna (1970) with indigo snakes (*Drymarchon corais*). Kleingenna trained (initial shaping of the behavior was required) three adult indigo snakes to press a wire in order to receive a water reward. The number of times a snake pressed the wire with a portion of its body increased over time. Eventually, the snakes learned to press and hold the wire to obtain a water reward. The snakes had clearly learned "how to use" Kleingenna's device.

An ecologically relevant example of operant conditioning is described by Greene (2003). An adult male Blacktail rattlesnake (*Crotalus molossus*) was observed setting up an ambush adjacent to a rodent trail. The view of the trail was obstructed by overhanging vegetation. In a series of movements similar in topography to those of male-male combat (Gillingham et al., 1983), the snake extended the anterior portion of its body and moved the overhanging vegetation out of the way. I do not consider this species-typical

behavior and is best considered as non-arbitrary behavior under the control of discriminative stimuli (i.e., the overhanging vegetation).

Taxonomies of learning

In addition to problems with the definitions of learning phenomena, it is important to be aware that there is no generally accepted taxonomy of learning. The importance of taxonomy and the relationships among entities is well known. For example, Murphy et al. (2002) describe the evolutionary relationships among rattlesnake species. An understanding of the relationships among the different types of learning is equally important. It is interesting to note how much effort was devoted to learning taxonomies in the early learning literature and how discussions of learning taxonomies have all but disappeared from the contemporary learning literature. As Bitterman (1962) noted over 40 years ago, “Classification is not merely a matter of taste” (p. 81).

Tulving (1985) describes six ways in which a classification scheme can advance the field of learning. These include providing theoretical structure to the design and analysis of experiments; replacing general categories such as classical and operant conditioning with detailed descriptions of the procedures; novel procedures and results can be described easily in terms of the amount of deviation from specified categories. I would suggest that those interested in conducting learning research with rattlesnakes attempt to link their procedure with one of the classification schemes.

Several taxonomies have been proposed: Dyal and Corning (1973); Gormezano and Kehoe (1975) for classical conditioning; Woods (1974) for instrumental and operant conditioning. Woods’ (1974) classification of instrumental conditioning identifies 16

categories of conditioning based on the presence or absence of a discriminative stimulus and the desirability of the reward.

Developing the taxonomy of learning will require agreement among psychologists on the definitions of different forms of learning. These definitions will likely require data on the different types of procedures, stimuli used, reinforcement contingencies and other parameters associated with experimental design. Developing the taxonomy of learning will also require a comparative approach, in which the performance of many different species, on many different learning tasks, is optimized onto a phylogeny. Then, the way the different types of learning are related can be hypothesized based on similarities in experimental procedures and their occurrence in related species.

The reporting of individual data

Most studies of learning involve the reporting of group data. Many examples can be found in the herpetological literature. Consider the study by Scudder et al. (1992), in which one of the explicit questions addressed was “how does [chemosensory searching] change with feeding experience?” Data are often presented as the percentage of animals responding on each trial. In other cases, data are presented as group means (as was done by Scudder et al., 1992). Group data do not give the shape of individual learning curves nor information about the variation among animals. Moreover, the number of records discarded from a test population is rarely reported. Without such data, it is difficult to know how many animals from a given population do indeed learn. Thus, the reliance on group data could lead to statements about species characteristics that are not reliable or valid (Hirsch and Holliday, 1988).

Many of the papers reviewed for this chapter were based on noticeably small sample sizes or the data were pooled from several species (e.g. Duvall et al., 1978). Small sample sizes are not inherently “bad” but underscore the importance of using single subject designs instead of group designs. Additionally, statistical analyses based on small samples often have low statistical power (ability to detect treatment effects).

In studies that indicate treatment effects, statistical power is generally not important, except in instances where treatment effects may be driven by one or two outliers in one of the groups being compared. This would lead to a Type I error (finding a treatment effect when one does not exist) and underscores the importance of including individual data, though outliers might be detected in group data by large standard deviations or standard errors in one or more of the treatment groups (I have encountered this phenomenon in some of my habituation studies).

In a single subject design, each animal serves as its own control. Repeated measures analysis in the single subject design also increases statistical power in situations of small sample size (Winer et al., 1991). Measuring a small sample of subjects multiple times and applying a completely randomized design constitutes pseudoreplication (the observations are not independent) and should be avoided. An alternative to repeated measures designs is the permutation test (Siegel and Castellan 1988). These tests are as powerful as their parametric equivalents (Siegel and Castellan 1988). Finally, one of the non-parametric tests based on ranks (e.g., Mann-Whitney U) may be more appropriate for behavioral data since many of the assumptions of parametric statistics may be violated. Many of these rank-based tests are nearly as powerful as their parametric equivalent (Siegel and Castellan 1988).

Cognitive Explanations of Learning

The trend toward interpreting learning in terms of “representations,” “cognitive maps,” and other language borrowed from the vocabulary of human information processing may be unwarranted for reptiles at this time. It is often overlooked that there are challenges to use of cognitive concepts when applied to the learning of animals (Amsel, 1989; Skinner, 1989). Even if some vertebrate species possess cognitive structures, the necessary experiments have not been performed to determine whether reptiles also possess such structures. For example, seemingly, complex responses to stimuli presented simultaneously or changes in reinforcement strength are explainable without recourse to cognitive constructs (Couvillon and Bitterman, 1984). The same is also true with avoidance learning (Abramson et al., 1988). It is interesting to consider that since over 95%-97% of all animals are invertebrates, and invertebrates do not have cognition, the importance of cognition is really not great. This is especially so when considering that most vertebrate behavior is not cognitive, either.

The rise in cognitive interpretations of animal behavior is alarming. Students are not being trained in traditional learning methodology and few professors have a grasp of the early learning literature. As surprising as this may sound, the word “Behavior” has all but disappeared from glossaries of introductory textbooks in zoology, biology, and psychology. In a casual survey of 138 introductory texts from the fields of psychology, biology, and zoology, only 38 (27.5%) of those contained a definition of behavior in the glossary. I view the absence of the word behavior as problematic given the rise of cognitive approaches to the study of behavior (Amsel, 1989). Traditional behavioral

issues are being tossed aside and all but forgotten by a new generation of students (Abramson, 1994; 1997).

It is not a fantasy to imagine that future discussions of such important areas as classical and operant behavior will be distorted and subsequently forgotten. Such a trend is already documented for texts used in advanced courses on the psychology of learning (Coleman et al., 2000). Similarly, Sheldon (2002) has shown the inconsistency with which current introductory psychology texts portray operant conditioning and associated issues.

When attempting to interpret rattlesnake learning in terms of cognitive constructs, it is important to recall C. Lloyd Morgan's canon: "In no case may I interpret an action as the outcome of the exercise of a higher psychical faculty, if it can be interpreted as the outcome of the exercise of one which stands lower in the psychological scale" (Morgan, 1894/1977, p. 53). If the behavior of rattlesnakes can be explained using classical and operant conditioning as a basis, then it is not necessary to suggest cognitive mechanism.

I venture to guess that most cognitive psychologists cannot define cognition and even fewer would know that Edward Chase Tolman was perhaps the first cognitive psychologist but was so within a behaviorist framework (Tolman, 1932). It is also not generally known that "cognitive" approaches were a central theme of many early behaviorists. For example, Clark Hull's papers in *Psychological Review* reveals titles such as "Knowledge and purpose as habit mechanism" and "Goal attraction and directing ideas conceived as habit phenomena" (Amsel and Rashotte, 1984).

The same is true of Edward L. Thorndike (Thorndike, 1949) and B. F. Skinner, both of whom were concerned with "cognitive" processes. I would ask my readers to

consider that if all animals have “cognition” why bother having an evolution of learning? Moreover, it is easily overlooked that the rise of cognitive psychology as applied to animals could only have occurred against a background of the large behavioral literature conducted within a traditional learning framework.

Before one can conclude that snakes have “cognitive” processes, a significant database must be developed based on traditional learning procedures. Clearly, the five or so rattlesnake learning studies are insufficient to warrant any cognitive interpretation. Without adequate data, interpreting the learning of rattlesnakes would be as ridiculous as interpreting the behavior of an ant in terms of Jungian archetypes.

In the previous sections, I have summarized the various types of learning and some of the issues that rattlesnake researchers must confront before undertaking learning studies. These issues include lack of consistency in behavioral definitions, no generally accepted taxonomy of learning, the lack of individual data, and the inappropriateness of cognitive explanations of behavior. I also touched briefly upon the lack of training of students in traditional areas of learning. In the next section, I will review the literature on learning in rattlesnakes.

Non-associative Learning

Habituation

Habituation refers to the reduction in responding to a stimulus as it is repeated. For a decline in responsiveness to be considered an instance of non-associative learning, it must be determined that sensory adaptation and motor fatigue do not exert an influence. Generally, two types of habituation are recognized: short-term and long term. The

principal difference is length of memory. Studies of habituation show that it has several characteristics, including the following (Thompson and Spencer, 1966):

1. The more rapid the rate of stimulation, the faster the habituation.
2. The weaker the stimulus, the faster the habituation.
3. Habituation to one stimulus produces habituation to similar stimuli (generalization).
4. Withholding the stimulus for a long period of time leads to recovery of the response (spontaneous recovery).
5. Habituation is a negative exponential function of the number of stimulus presentations.
6. Rate of habituation increases as the number of training sessions increases.
7. Habituation may continue beyond zero.
8. Presentation of a strong novel stimulus results in the return of the habituated response (dishabituation).
9. Continued application of a dishabituation stimulus results in habituation of dishabituation.

Sensitization

Sensitization refers to the augmentation of a response to a stimulus. It is the opposite of habituation and refers to an increase in the frequency or probability of a response. Also, as with habituation, two types of sensitization: long-term and short-term. Sensitization shows several characteristics including the following:

1. The stronger the stimulus is, the greater the probability that sensitization will be produced.
2. Sensitization to one stimulus produces sensitization to similar stimuli.
3. Repeated presentations of the sensitizing stimulus tend to diminish its effect.

In contrast to most other types of behavior modification, the definitions of habituation and sensitization are remarkably consistent from experimenter to experimenter. If, for example, a group of psychologists, zoologists, and herpetologists were asked to define habituation, they would probably provide a definition similar to that offered by Harris (1943), in which habituation is defined as a "response decrement as a result of repeated stimulation" (p. 385). The same is true of sensitization, which Teyler (1984) has defined as "an augmentation of a response to a stimulus" (p. 176).

What is also remarkable about habituation and sensitization is that they are ubiquitous throughout the animal kingdom. They even appear, for instance, in experiments in which the "animals" consist of single cells or isolated ganglia. In terms of the evolution of learning mechanisms, habituation and sensitization may well be the most basic process for behavior modification.

The study of habituation and sensitization is interesting for a number of reasons. First, habituation and sensitization experiments are easy to perform – whether the animal is freely moving or restrained, semi-intact, or "missing"-save for an isolated portion of a nervous system. Second, habituation and sensitization share many properties with more complex learning phenomena, such as the ability of the response to recover over time; creating new behavior patterns; improvement in performance over successive sessions; and sensitivity to such training parameters as intensity, frequency, and pattern of stimulation. Third, there are several well-defined characteristics that can be compared across species. The characteristics of habituation and sensitization can be compared not only across species but also across the successive stages of a research preparation, as when I study sensitization in the intact animal, a semi-intact specimen, and progress to

isolated portions of the nervous system. It should be obvious that results obtained at each stage of a preparation can also be compared with analogous stages from other research preparations.

The significance of habituation and sensitization should not be underestimated. Though not as glamorous as the behavior change associated with classical or operant conditioning, their behavioral manifestations are just as adaptive. In addition, for many animals, this represents the only type of behavior modification possible. Habituation and sensitization increase the chances of survival and reproduction by minimizing wasted energy and by reducing the occurrence of maladaptive behavior.

Controls.--Before a decrease in responsiveness can be attributed to learning, several alternative explanations must be ruled out. The two most important are effector fatigue and sensory adaptation. The base rate of responding must also be considered.

A primary source of confusion in habituation experiments is the decrease in responsiveness in sensory organs subjected to intense or prolonged periods of stimulation. Two major procedures have been used to rule out the influence of sensory adaptation. First, one can select an intertrial interval – the time between presentations of the habituation stimulus – that is long enough to allow the effect of adaptation to wear off. Second, if long intertrial intervals are not practical, a test trial procedure can be substituted in which habituation is assessed not during training but during test trials administered sometime after training. Select a time interval between training and testing that is long enough for adaptation to dissipate. Determining the duration of this interval will involve some guess work and knowledge of the physiology of the response.

A second source of error in habituation experiments is that the effector mechanisms responsible for the expression of the response are not able to function. To separate the effects of fatigue from habituation, it is common to give the animal a test trial(s) using a second stimulus that elicits the target. If there is a response to this other stimulus, and a response to the reintroduction of the original training stimulus, the effect of fatigue may be ruled out. Place and Abramson (unpubl. data), for example, were able to demonstrate that their snakes were still able to rattle by prodding them with a snake hook. Dishabituation is probably the most widely used control to assess the influence of fatigue in habituation experiments.

A third source of error is that many responses that habituate occur without any noticeable stimulation. This is common especially in situations where the response that is habituated involves some type of movement (such as eye blink) and general activity. The only way to account for this is by determining the base rate of occurrence of the response being measured.

Before one can conduct and accurately interpret the results of any habituation experiment, it is important to know the rate, duration, and temporal pattern of the response that is to be habituated or sensitized. It is also important to determine if the change in behavior is the result of maturation or development. Given the rapid progress in the life cycles of many animals, such a possibility must be taken seriously. To establish a base rate of responding, add a control group to the experimental design that is placed in the training situation but not given any habituation training. Record the data as one would for a training run.

Review of the literature.--As I have noted, there are no formal studies of habituation and sensitization in rattlesnakes except for a study of habituation done by Place and Abramson (see chapter IV). They showed that the rattle response exhibits both short-term and long-term habituation. A rattle response was elicited in *Crotalus atrox* at 5-min intervals in an automated apparatus (Place et al., in press) until the response failed to occur in 10 consecutive trials. The same procedure was performed on four consecutive days. It is important to note that a 10-trial criterion is very strict. A study of habituation of defensive head movements in garter snakes used a criterion of four consecutive no response trials (Hampton and Gillingham, 1989). Appropriate controls for effector fatigue and sensory adaptation were used in the Place and Abramson (unpubl. data) study. These effects were ruled out by administering a prod to the snake with a snake hook, after it reached criterion, and then testing for a response with an additional test trial.

There are several studies in the rattlesnake literature that either implicitly or explicitly indicate habituation or sensitization (Table 1). Much of the work by Chiszar and colleagues on strike induced chemosensory searching (SICS) clearly suggests habituation (Table 1). However, because the primary goal of these studies was not to demonstrate habituation, appropriate controls for an unambiguous interpretation are lacking.

Chiszar, et al. (1976) studied changes in rate of tongue flicking (TF) in *C. viridis*, *Sistrurus catenatus edwardsi*, and *S. c. tergeminus* in three different environments. The environments were the home cage, an open-field, and an open-field with rodent odors present. They found a significant decrease in TF over 30 minutes in all species, and differences in overall rates of TF between species and subspecies. Furthermore, they

found differences in TF between the home cage and the two open-field conditions. The open-field conditions did not differ from one another. This study underscores the importance of species and subspecies comparisons and the potential influence of environmental variables. It is not known if the decrease in TF is the result of habituation, effector fatigue, or sensory adaptation because a dishabituating stimulus and subsequent test trial were not administered.

Scudder and Chiszar (1977) examined the defensive responses and TF of *C. viridis* and *S.c. tergeminus* in the presence of six different visual stimuli in the subject's home cage and in an open field. The defensive response was scored on an ordinal scale from 1-6, with 1 being no response and 6 being the most defensive. The six stimuli tested were stationary, taxidermy-mounted coyote (*Canis latrans*) and mink (*Mustela vison*), a live dog (*Canis familiaris*), a stationary human face, a moving human face, and a moving curtain control. Both species were more responsive in the open field than in their home cage, but *C. viridis* exhibited a higher defensive response score and more TFs than *S. c. tergeminus* in both conditions.

Table 1.1 Essential elements in studies of habituation and sensitization. Note most of the studies cited were not intended to demonstrate habituation and sensitization and lacked appropriate control procedures

Subject variables

Species comparisons (Chiszar et al., 1976; Scudder and Chiszar, 1977)

Sex (Place and Abramson, unpubl. data)

Age (Not systematically tested)

Free-ranging animal (Not systematically tested)

Semi-intact or isolated preparations (Not systematically tested)

Rearing environment

Isolated or group (Not systematically tested)

Small or large enclosure (Marmie et al., 1990)

Wild or long-term captive (Chiszar et al., 1985; Chiszar et al., 1999)

Environmental variables

Characteristics of apparatus (Chiszar, et al., 1976; Chiszar et al., 1978)

Naturalistic vs. laboratory environments (Not systematically tested)

Temperature (Not systematically tested)

Seasonal variability (Not systematically tested)

Ecological manipulations (Not systematically tested)

Response measures

Amplitude or intensity of response (Not systematically tested)

Frequency of response (Most studies using tongue flicks as a dependent variable)

Latency of response (Place and Abramson, unpubl. data)

Duration of response (Place and Abramson, unpubl. data)

Total number of responses (Place and Abramson, unpubl. data)

Rate of habituation (Place and Abramson, unpubl. data)

Individual differences (Place and Abramson, unpubl. data)

Stimulus variables

Single stimuli (Most studies that indicate habituation in rattlesnakes)

Multiple stimuli (Scudder and Chiszar 1977; Chiszar et al., 1978; Melcer et al., 1988)

Concurrent stimuli (Not systematically tested)

Controls

Base rate of responding (Chiszar et al., 1982; Melcer et al., 1988)

Sensory adaptation (Place and Abramson, unpubl. data)

Effector fatigue (Place and Abramson, unpubl. data)

General experience (Not tested)

Interestingly, in the home cage condition, for the defensive response score, there was a significant stimulus by interval interaction, indicating a decrease in responsiveness for some stimuli but not others. Additionally, TF did result in a significant main effect of interval, which indicates a decrease in TF between intervals. In the open-field condition, there was a significant stimulus by interval interaction for both response score and TF. In all cases, the dog and moving face elicited higher responses. Habituation in this study is difficult to assess because, essentially, only two trials were carried out and no

dishabituation stimulus was administered. Nonetheless, the importance of testing multiple stimuli and making species comparisons were emphasized.

In a study similar to that of Chiszar et al. (1976), Chiszar et al. (1978) found that the rate of TF by *C. viridis* differed between three environmental conditions and the home cage. The three environmental conditions were an open field, a cage inhabited by a hognose snake, and a cage inhabited by an ophiophagus kingsnake. Tongue flick rates declined significantly during the 10-minute testing period in all four environments. However, this decrease in response can be attributed to habituation, effector fatigue, or sensory adaptation, because a dishabituating stimulus control was not performed.

A study performed by Chiszar et al. (1982) that assessed the occurrence of strike-induced chemosensory searching (SICS) in five genera of viperid snakes indicated that sensitization of TF occurred in the 12 rattlesnakes tested following a strike on a live mouse. A gradual increase in TF was present up to ten minutes following the strike. Habituation was evident in the same group of snakes in the no-strike condition, in which a mouse was suspended in the cage out of strike range for 3-sec. Data from eight different rattlesnake species were pooled to generate the sensitization curve and no individual data were reported. This study shows that habituation and sensitization can potentially occur in the same group of animals, and that these changes in response over time depend on initial conditions. A learning interpretation must be viewed with caution since there were no controls and individual results are not reported. It is possible that one or two species in the group of 12 had much greater or lower responses than the others and thus were driving the shape of the habituation and sensitization curves.

Strike-induced chemosensory searching generally lasts for about two hours (e.g., Marmie et al., 1990) before tongue flicking (TF) rates return to normal and may be characterized as sensitization, or alternatively, the return to baseline levels may be habituation. I recommend, in studies of SICS, that a dishabituating stimulus (e.g., gently prodding snakes with a hook) be applied when TF rates return to baseline so sensory adaptation and motor fatigue can be ruled out as possible explanations for decreased TF.

Chiszar et al. (1985) studied the influence of long-term captivity on the duration of SICS and found no effect. Habituation was evident in the strike condition, although the TF data are not reported after 120 min. The repeated stimulus in this situation is interpreted as the presence of some odor stimulus. The average time to return to baseline was 142.5 min, though the shape of the habituation curve was not presented. The decline back to baseline can be attributed to habituation, effector fatigue, or sensory adaptation. Since a dishabituating stimulus was not presented, these effects cannot be separated. This study highlights the importance of testing the influence of long-term captivity on behavioral responses. Similar studies need to be done directly assessing habituation using appropriate control procedures.

Melcer et al. (1988) performed two experiments illustrating the utility of using novel perfume odors as potential stimuli. Perfume did not influence ingestion behavior. In a second experiment testing the influence of perfume odor on SICS following a strike, an increased TF was observed that remained elevated until the end of the 20-minute testing period. In the no-strike condition, habituation of TF was evident over the 20-minute testing period to both perfumes tested, along with the water control. Once again, no control for habituation or sensitization was applied in these experiments, so

habituation should be attributed with caution. This is an important study because it illustrates the utility of perfume as useful potential discriminative stimuli. Unfortunately, the results are based on data pooled over four *C. horridus* and six *C. viridis*. While they report no significant differences between species, this should be viewed cautiously, given the small sample sizes for each species.

Marmie et al. (1990) performed two experiments to determine the effect of rearing environment and captivity on exploratory behavior and SICS in *C. enyo*. Individual data were not presented, but TF did decline over 150 minutes, suggesting habituation, but controls were lacking to confirm this. Interestingly, rates of TF differed between wild and captive rattlesnakes, but not between captive snakes reared in different sized enclosures. When considering the duration of SICS, the importance of individual data becomes evident. Marmie et al. (1990) noted that 2 of 4 wild, and 7 of 12 captive snakes, still had TF rates above baseline after 150 minutes, indicating probable individual differences in the shapes of their response curves.

Chiszar et al. (1999a) described the complete recovery of SICS in six specimens of *C. oreganus* rescued from substandard living conditions. Their figure 1 clearly illustrates habituation of the TF response over 30 minutes. However, effector fatigue and sensory adaptation cannot be ruled out as explanations for the decline in response. This is another study that highlights the importance of testing the impact of environmental variables and prior history on learning.

As illustrated by the studies I have reviewed, habituation in rattlesnakes is a fruitful area of research. In addition to exploration of training variables such as intertrial interval and stimulus intensity, environmental variables (Table 1) need to be tested.

These variables include apparatus characteristics such as size of the open field or light intensity within the open field. The work of Chiszar et al. (1976, 1978) is a good first step in assessing these effects. Naturalistic environments also need to be tested, though I recommend a thorough understanding of laboratory-based habituation experiments before field studies are implemented. Since rattlesnakes are ectotherms, temperature probably has a profound effect on learning and other behavioral processes. The effect temperature has on habituation and sensitization in rattlesnakes has not been tested.

Very few response measures have been studied in rattlesnakes (Table 1). This is surprising, given that rattlesnakes exhibit several more easily quantifiable responses than other snake taxa. The large number of studies discussed above that examined TF responses are commendable, but rattling behavior lends itself to quantification better than simple TF rates. Other responses that can be measured include presence/absence of rattling, latency to rattle, duration of rattling, and the intensity of the rattle sound. Once a better understanding of these measures is acquired, additional studies into rates of habituation and individual differences can be explored. Especially exciting is the possibility of combining neurophysiological and behavioral data utilizing semi-intact and isolated preparations of the vomeronasal organ system. The vomeronasal organ system is perhaps the best understood sensory system in snakes, from both an intact behavioral perspective as well as a simple systems perspective (Halpern, 1992).

Stimulus variables also need to be assessed (Table 1). Testing TF responses to various odors as Melcer et al. (1988) and Chiszar et al. (1976, 1978) have done is a good first step. Experiments utilizing multiple and concurrent stimuli need to be carried out. Determining what stimuli elicit rattling behavior needs to be determined. The

experiments by Place and Abramson (unpubl. data) on habituation of the rattle response made use of visual, auditory, and vibrational cues, simultaneously. It is unknown if any one of these stimuli in isolation elicits rattling, although Scdder and Chiszar (1977) suggest that visual cues alone elicit a generalized defensive response.

The work on habituation and sensitization in rattlesnakes barely scratches the surface of what can be accomplished. There are no studies, for example, investigating such habituation effects as generalization, spontaneous recovery, or habituation of dishabituation. These studies are simple to perform and can provide a stable database for expansion into more ecologically based problems of interest to rattlesnake biologists. In addition to the lack of studies investigating parameters known to be influenced by habituation, there are no studies investigating the role of subject variables in habituation. These variables include sex, developmental stage, and previous experience.

Associative Learning

Classical Conditioning

Classical conditioning refers to the modification of behavior in which an originally neutral stimulus – known as a conditioned stimulus (CS) – is paired with a second stimulus that elicits a particular response – known as the unconditioned stimulus (US). The response that the US elicits is known as the unconditioned response (UR) and the response to the CS is known as the conditioned response (CR). The CR and UR are generally similar and derived from the same response system. The CS is generally followed or slightly overlapped in time by the US. There are two major classes of classical conditioning experiment. Appetitive classical conditioning is demonstrated if a rattlesnake is trained to extend its tongue to a novel odor (CS) that is followed by food

(US = food odor). Aversive (or defensive conditioning) is demonstrated if a rattlesnake is trained to rattle its tail (a defensive behavior) in the presence of the novel odor (CS) that is followed by a brief aversive event (US) such as electric shock. Studies of classical conditioning show that it has several characteristics, including the following:

1. In general, the more intense the CS is, the greater the effectiveness of the training.
2. In general, the more intense the US is, the greater the effectiveness of the training.
3. In general, the shorter the interval is between the CS and the US, the greater the effectiveness of the training.
4. In general, the more pairings there are of the CS and the US, the greater the effectiveness of the training.
5. When the US no longer follows the CS, the conditioned response gradually becomes weaker over time and eventually stops occurring.
6. When a conditioned response has been established to a particular CS, stimuli similar to the CS may elicit the response.

Classical conditioning is an example of associative learning in which the behavior of the animal is altered by the pairing of stimuli, one of which is effective in eliciting a biologically important reflex. In a broader sense, classical conditioning is a family of methods for the acquisition of associations between two or more stimuli or between stimuli and responses (see, for example, Instrumental Approach Design in the previous section on inconsistencies in definitions). Classical conditioning is generally thought to represent the most basic of the associative learning mechanisms (Razran, 1971).

Before it can be concluded that the appearance of a CR is the result of the formation of an association between the CS and US, several alternative explanations must be eliminated, especially pseudoconditioning.

Controls.--If a US is presented over the course of several trials and then a CS is introduced, a response resembling that elicited by the US will often occur to the first presentation of the CS. This phenomenon is called psuedoconditioning.

Pseuoconditioning is not considered a CR because it was not the result of CS US pairings. Pseudoconditioning occurs because the intital presentations of the US result in a general excitation in the subject. To determine if pseudoconditioning occurs, one should include a control group receiving the same number of CSs and USs as the experimental group, but presentation of the CS is separated from US presentation by an intertrial interval. If this unpaired group shows an increase in response to the CS over several trials it is a result of pseudoconditioning because the CS and US were not presented in association.

It is customary when employing an unpaired control to keep the training period constant between experimental and unpaired control groups. This is accomplished by using an intertrial interval that is half that used in the paired group (Abramson, 1994). For example, if the intertrial interval is one minute in the experimental group, it will be 30 seconds in the unpaired control group. Classical conditioning would be demonstrated if responses to the CS are greater in the group receiving paired CS-US presentations than in the group receiving unpaired presentations.

A second way to estimate pseudoconditioning is to train an animal to discriminate between two CSs, one of which is paired with the US. Classical conditioning would be demonstrated if the animal is able to discriminate between them.

Pseudoconditioning should not be considered merely a control in classical conditioning experiments. The behavior modifications produced by pseudoconditioning are as important as those produced by classical conditioning and deserve additional attention by behavioral scientists (e.g., Wickens and Wickens, 1942; Razran, 1971). How pseudoconditioned responses are formed is still a mystery. Such responses may reflect an internal sensitization process that causes a particular external stimulus to trigger a response; alternatively, such responses may reflect a similarity between the US and the CS (Mackintosh, 1974).

Review of the literature.-- I was surprised to find that no studies of classical conditioning are reported in the literature on rattlesnakes despite the interest in classical conditioning in traditional learning theory and the use of classical conditioning as a bioassay for many types of animals (e.g., Abramson et al., 2004). This is a wide-open area where many contributions can be made.

An excellent place to start would be to modify the technique of Melcer et al. (1988) and Melcer and Chiszar (1989a,b) to test for latent inhibition and blocking. In a hypothetical latent inhibition experiment, a number, say 15, of presentations of Jungle Gardenia perfume alone could be followed by 20 presentations of perfume together with a mouse. If latent inhibition is occurring, an initial lag in tongue flicking will be followed by a gradual increase in tongue flicking to an asymptote. The results of the inhibited group should be compared to the results of a paired only group. For this procedure to

work, the number of tongue flicks elicited by the perfume must be lower than the number of tongue flick elicited by the mouse alone. Moreover, the number of training trials is only a suggestion. The necessary experiments have not been performed for us to know how many training trials may be necessary. For a more thorough discussion of latent inhibition, see Lubow (1973)

These techniques can also be applied to blocking designs. A hypothetical experiment might proceed as follows. Initially Jungle Gardenia perfume is paired with mouse odor each on a separate cotton swab for 15 trials. Then, a second neutral stimulus, say Halston perfume, is presented between the presentations of Jungle Gardenia and mouse odors for 15 trials. These pairings are then followed by one test trial in which the Halston is presented alone. If blocking occurs, the snake should not exhibit elevated TF rates because the Halston did not provide any new information about the mouse.

In contrast to habituation studies in rattlesnakes, there is no literature on classical conditioning. Such data are vitally important not only from what it can tell us about classical conditioning in rattlesnakes, but also for what it can tell us about how classical conditioning is used under natural conditions. Before such progress can be made scientists interested in rattlesnake behavior must devote resources to the study of classical conditioning. For example, what is the optimal CS-US interval in rattlesnake preparations; what is the effect of backward CS-US pairings on the acquisition of a classical conditioning response; do rattlesnakes exhibit inhibitory and excitatory classical conditioning? Equally important are applied questions. Can classical conditioning be used to test the influence of toxic environments on behavior? Such an approach has been profitable applied to honey bee behavior (Abramson et al., 2004). It is a well-known

practice for rattlesnake hunters to pour gasoline into rattlesnake dens. The effect of such a practice has received scant attention (Campbell et al., 1989). Moreover, classical conditioning can be used to test a variety of rattlesnake repellents and attractants.

Instrumental and operant conditioning

Instrumental and Operant Conditioning refer to the modification of behavior involving a participant's responses and the consequences of those responses. It may be helpful to conceptualize an operant and instrumental conditioning experiment as a classical conditioning experiment in which the sequence of stimuli and reward is controlled by the behavior of the subject. In contemporary usage, the terms instrumental and operant conditioning are used interchangeably. However, there are several differences in methods and procedures. For example, instrumental procedures usually involve discrete trials in which the investigator determines the amount of time between trials. In operant procedures, the subject determines the interval between responses. Another difference between instrumental and operant conditioning is the response variables measured. In instrumental conditioning variables such as latency and duration are recorded; whereas in operant conditioning the response rate is recorded.

When there is a positive relationship between a response and a desirable outcome, such as when a rattlesnake finds a bit of food at the end of a maze, the phenomenon is known as reward training. A special case of reward training is known as escape. In escape training, a response terminates an unwanted event and the reward is time away from the aversive event. For example, a rattlesnake may run through the maze to escape a predator. The reward in this situation is finding a compartment where it is safe from the predator. Alternatively, if the snake runs through the maze only to find the predator, it

eventually refuses to run the maze, as a result of punishment training. If raising the door of the maze causes the snake to run the maze to prevent the predator from being released, signaled avoidance training has occurred. The signal in this example is a compound of the noise and vibration associated with the opening of the door. In the final paradigm, if the snake remains motionless to obtain a reward omission training has occurred (Abramson, 1994). Studies of instrumental and operant conditioning show that they have several characteristics, including the following:

1. In general, the greater the amount and quality of the reward, the faster the acquisition.
2. In general, the greater the interval of time between response and reward, the slower the acquisition.
3. In general, the greater the motivation, the more vigorous the response.
4. In general, when reward no longer follows the response, the response gradually becomes weaker over time and eventually ceases.

Instrumental and operant conditioning are examples of associative learning in which the behavior of an animal is controlled by the consequences of its actions. These two types of conditioning are generally thought to be more complex than classical conditioning. One might characterize the difference by saying that classical conditioning describes how animal make associations between stimuli and instrumental and operant conditioning describe how animals associate stimuli with their own motor actions. In addition, instrumental and operant conditioning are thought to be more complex than classical conditioning because learning depends on the animal's own behavior and usually requires a more obviously new behavior. Despite these differences, instrumental

and operant conditioning share many properties with classical conditioning. These include extinction, spontaneous recovery, generalization, and discrimination.

Before it can be concluded that a change in behavior is the result of the formation between a response and reinforcer, several alternative explanations must be eliminated. The problem of control is not as great as in classical conditioning because the instrumental or operant response should be a behavior that is not in the repertoire of control animals. For example, if snakes are trained to press a lever for food and then placed in a general population of untrained snakes, it should be an easy matter to determine which animals are trained. In addition, many maze experiments have a built-in control by requiring the animal to make a discrimination between two stimuli or learn a series of complicated paths.

Controls.--As in classical conditioning, the primary worry is to separate true conditioning from pseudoconditioning. In this case, it must be ensured that the reinforcement does not produce a change in behavior resembling the index of instrumental or operant conditioning. As an example, let consider a study of the acquisition of runway performance in a snake with access to water as the reward. Over a series of 20 trials, the speed of the snake is observed to increase with training. Can I conclude that the increased speed means that the thirsty snake has associated the goal box with water? The answer is no. It is just as likely that the increased speed would have occurred without any reward in the goal box. Increased speed might also represent the energizing effect of exposure to water that is independent of the contingency between the response and the reward. To assess the amount of pseudoconditioning, a control group is necessary that occasionally receives water in a location other than the goal box. This is

similar to the unpaired group in a classical conditioning experiment. For example, snakes may occasionally get access to water in a separate enclosure and not in the goal box. It is also possible to assess pseudoconditioning by training the snake to discriminate between two stimuli, one of which is associated with reward.

Review of the literature.--The only instrumental/operant conditioning study performed with rattlesnakes is an unpublished dissertation by Mills (1970) that demonstrated instrumental escape behavior in two individuals (*Crotalus scutulatus* and *C. cerastes*). Mills attempted to demonstrate signaled avoidance using an infrared signal preceding subcutaneous shock. The restrained snake could avoid shock by moving its head laterally through a photogate. Avoidance was not achieved after 6000 trials over ten consecutive training days. It is important to note that the lateral head movement is not a typical rattlesnake defensive behavior. Bolles (1970) suggested that animals in escape and avoidance training would only learn responses that were specific to their species-typical defensive behavior. He called these species-specific defensive reactions (SSDR). In rattlesnakes, SSDRs should include movement, freezing, rattling, and striking.

Several studies have used visual, chemical, thermal, and tactile cues to investigate discrimination learning (Table 1.2) in rattlesnakes. Unfortunately, these studies did not indicate whether the learning was classical, instrumental, or operant. Possibilities include classical conditioning of associated stimuli or instrumental conditioning of discriminative stimuli and the appropriate response by the snake. I have placed these studies in the instrumental/operant section because they involve the animal moving toward the source of stimulation.

Table 1.2. Published studies utilizing rattlesnakes in which discrimination was inferred, but the mechanism of learning was undetermined. See text for additional discussion.

Species	Discriminative stimuli	Response	Reference
<i>C. horridus</i>	Methanol, prey extracts	TFAM ¹	Clark, 2004b,c
<i>C. horridus</i>	Not determined	Proximity,	Clark, 2004a
<i>C. viridis</i>	Blank, conspecific odor	Time allocation, TF ²	Scudder et al., 1988
	Blank, heterospecific odor	Time, TF	
	Blank, conspecific odor	Y-maze	
<i>C. durissus</i> , <i>C. enyo</i>	Large prey, small prey	Release, hold prey	Radcliff et al., 1980
<i>C. viridis</i>			
<i>C. terrificus</i> , <i>C. viridis</i>	envenomated mice,	Time, TF	Duvall et al., 1980
	non-envenomated mice,		
	nasoral region of rodent,		
	anogenital region of rodent		
<i>C. atrox</i>	envenomated mice,	TF	Chiszar et al., 1999
	non-envenomated mice		
<i>C. atrox</i> , <i>C. durissus</i> ,	envenomated, non-envenomated	Swallowing, TF	Duvall et al., 1978

<i>C. enyo</i> , <i>C. lepidus</i> ,	mice		
<i>C. mitchelli</i> , <i>C. viridis</i>			
<i>C. triseriatus</i> , <i>C. willardi</i> ,			
<i>C. pricei</i> , <i>Sistrurus</i>			
<i>catenatus</i>			
<i>C. viridis</i>	Clean cage, mouse soiled shavings,	Time moving, TF,	Duvall et al., 1990
	Live mice, soiled shavings	time in ambush,	
		mouth gapes, nudges,	
		strikes, location in	
		apparatus	
<i>C. viridis</i> , <i>C. enyo</i> ,	Jungle gardenia perfume,	swallowing, TF,	Melcer and Chiszar, 1989a
<i>C. horridus</i> , <i>C. atrox</i> ,	Tea Rose perfume, Halston,		
<i>C. adamanteus</i> , <i>C. ruber</i> ,	perfume, cinnamon fed prey,		
<i>S. miliarius</i>	cocoa fed prey, distilled water		
<i>C. viridis</i>	Distilled water, jungle gardenia	time oriented toward,	Melcer and Chiszar, 1989b
		TF	
<i>C. viridis</i>	Condom containing rat,	TF	Chiszar et al., 1991

	condom containing water-soaked cotton balls		
<i>C. viridis</i>	Presence/absence of prey	TF	Kandler and Chiszar, 1986
<i>C. viridis</i>	small, medium, large mice	total venom mass, Strike distance, strike duration, TTD ³ , anterior strike	Hayes, 1995
<i>C. oreganus</i>	small and large mice	Strike latency, number of strikes, strike distance, site of fang penetration, contact duration, total venom mass, venom dose, TTD, death rate	Hayes et al., 1995

¹TFAM = tongue flick ambush score, ²TF = tongue flicks, ³TTD = time to death of prey

Duvall et al. (1978) performed a discrimination study in which 25 rattlesnakes representing 10 species were tested several times each, in one of two conditions. The first condition was a “strike” condition in which a snake was allowed to strike a live mouse immediately prior to the discrimination task. The second condition was a “non-strike” condition in which the snake was allowed to observe a live rodent, but was not allowed to strike it. Dead mice were used in the discrimination task. A dead envenomated mouse was placed in the snake’s enclosure simultaneously with another pre-killed rodent and the snake was allowed to select and consume one of the rodents. In the strike condition, 71% of trials resulted in a subject choosing one of the mice, whereas, in the no strike condition, 79% the trials resulted in no selection. In the strike trials that resulted in a choice, most (69%) choices were for the envenomated mice. It is impossible to know if the discrimination of the envenomated prey was learned or innate. The subjects had 4 or 5 opportunities to strike, choose and swallow prey, but the consequence of choosing either of the mice was the same.

Duvall et al. (1978) offer four explanations. These are, 1) innate attraction to changes in mouse odor or taste as a result of envenomation, 2) innate attraction to residual saliva or venom from the snake’s attack on the surface of the rodent, 3) innate preference for mouse alarm factors, and 4) memory of the rodent’s odor or taste experienced during the strike. A fifth alternative is possible in that the snakes may be “prepared” or have an innate capacity to remember cues associated with rodents that have been struck. To implicate memory (i.e., learning) pseudoconditioning must be ruled out by utilizing a control group that gets the cues from the envenomated prey separated from the consequence of being able to consume the rodent. Alternatively, discriminative

stimuli can be used in a single subject design, in which two neutral stimuli are used, and only one of them is paired with the consequence of being able to consume the rodent. If learning occurred, selection of the rodent associated with the positive discriminative stimulus will increase.

In a follow-up study, Duvall et al. (1980) performed five discrimination experiments. In experiment one, six *C. terrificus* and six *C. viridis* were used. The procedure was similar to that of Duvall et al. (1978) except there was no “no strike” condition. The envenomated or non-envenomated mice were placed in plastic mesh bags fastened to a raised platform. Envenomated mice were envenomated by the test subject. Each snake was tested in one 10-minute trial. Discrimination was determined by TF and the percentage of time spent gazing toward the two bags.

The results indicated that both species spent significantly longer gaze time toward the bag containing the envenomated mouse. *Crotalus terrificus* also exhibited significant numbers of TF toward an envenomated mouse, whereas *C. viridis* did not differ.

Experiment two was similar to experiment one, except that the envenomated mice were envenomated by a conspecific. Both species spent significantly more time investigating the mouse envenomated by a conspecific than the non-envenomated mouse. *Crotalus terrificus* did not exhibit significant differences in the number of TF directed toward either mouse, though the total number of TF did approach significance. Low statistical power is probably responsible for not finding a significance difference. *Crotalus viridis* exhibited significant differences in the number of TF directed toward the envenomated mouse.

In experiment three, specific aspects of the predatory sequence were assessed in finer detail. They recorded the number of TF directed toward the nasal-oral versus the anogenital region of an envenomated mouse, the number of sweeps, initial latency to make contact with the tongue, which end of the mouse was grasped first by the snake, which end of the mouse to first receive swallowing movements, latency to begin swallowing, and duration of the swallowing process. Both species showed no difference in the number of TF directed toward either end of the envenomated mouse. *Crotalus viridis* exhibited a specific preference for headfirst ingestion, but *C. terrificus* did not. When data from both species were pooled, the headfirst preference was significant.

Experiment four was similar to experiment three, except the envenomated mouse was placed in a plastic mesh bag to prevent swallowing. Thus all subjects were tested for the same duration. Experiment three allowed subjects to swallow the prey, so test length was different for each individual. The number of sweeps, the percentage of TF oriented toward the nasal-oral region versus the anogenital region, and the frequency of attempts to grasp the hind and head ends of the bag were recorded. Both species directed more TF toward the head end of the mouse and made more attempts to grasp the head end of the bag. Snakes also made more TF in this experiment than in experiment three.

Experiment five tested the notion that odors associated with the nasal-oral region of an envenomated mouse are used in discriminating between the head and tail ends of the prey. Snakes were allowed to strike a live mouse. The envenomated mouse was switched with a nitrogen gas killed rodent and placed in the wire mesh bag. The dependent variables were the same as in experiment four. No differences were found in

any of the responses scored. The authors suggest that odors related to envenomation are emitted from the nasal-oral region of the rodent.

While the authors did not imply learning was involved in these experiments it is difficult to know because neutral stimuli were not associated with the envenomated and non-envenomated mice, nor with the head and tail ends of the prey. “Discrimination” could easily be the result of innate preferences or sensitization. Moreover, each subject was only tested in a single trial. I would expect that learning be developed over several trials. A third limitation hampering a learning interpretation is that prior to the experiment, each subject had fed on mice twice per week for at least six months prior to testing. They may have learned discriminative cues associated with handling and swallowing during the period preceding the experiments. This prior experience underscores the importance of using neutral stimuli in studies of discrimination. Additionally, statistical power is an issue in these experiments. The sample sizes were small and the same snakes were used in all five experiments. While this does not strictly constitute pseudoreplication, the possibility that experience carried over from experiment to experiment must be taken seriously. Surprising, Duvall et al, considered all of their observations as statistically independent.

Radcliffe et al. (1980) assessed how three species of *Crotalus* handle rodent prey of different sizes immediately following a strike. Eight *C. enyo*, five *C. viridis*, and six *C. durissus* were offered either a large or small live mouse during their regularly scheduled feeding. Presentation was counter balanced so half received the large first and the small second, while the other half received the small first and the large second. The rodent was presented by suspending it in the snake’s home enclosure by the tail with long

forceps. The dependent variable was whether the snake released or held the prey following a strike. Data were pooled across species. Holding small rodents occurred more often than holding large rodents. Interspecific comparisons revealed a difference in holding propensity between *C. enyo* and the other two species studied.

These conclusions should be viewed with caution because of the small sample sizes used. Radcliffe et al. (1980) suggested that snakes discriminate between large and small prey during the strike and decide at that moment to release or hold the prey. They were uncertain as to what cues were used to discriminate between large and small prey, but they cited Gans (1966) who suggested that prey mass may be inferred through the biomechanical consequences of striking large versus small prey. During the head retraction phase of the strike, small prey are easily lifted from the substrate, while large prey cause the snake's trunk to lift off of the substrate. Radcliffe et al. (1980) suggest an experiment in which models or live prey of similar visual, thermal, and chemical constitution, but differing mass, be presented to snakes and the decision to hold or release be recorded. If holding occurs in a mass dependent fashion, then, they contend, the decision is made during or after the strike, rather than before it. This is essentially the experiment they performed, although the thermal cues of their subjects probably differed between large and small rodents. Snakes hold their prey to reduce searching time. If learning is involved in the decision-making process, one should be able to reverse the consequences of the situation in which a snake holds and releases its prey. For example, one should be able to train a group of snakes to hold large prey by allowing it more rapid access to the rodent following a strike. A second group can be trained to hold small prey by making them wait longer before initiation of swallowing behavior. The result would

be that snakes can learn to hold large prey items and release small ones. If snakes cannot learn this task, holding small prey and releasing large prey is an innate behavior.

Kandler and Chiszar (1986) tested the spatial orientation of snakes following predatory strikes in the absence of prey chemical cues. Six *C. viridis* were tested in each of two conditions, strike and no strike (similar to Chiszar et al., 1982). Presentation of the mouse in each condition was counter balanced between subjects. Kandler and Chiszar recorded the number of TF directed to the side of the enclosure where the mouse was presented versus the side the mouse was not presented over a twenty-minute session. Analysis of variance revealed more tongue flicks to the mouse side than to the no mouse side in both conditions. These results should be considered with caution. Kandler and Chiszar did not test for position preferences in their snakes (but the stimulus arm was reandomized), so it is unknown if the orientation to the mouse side is a result of the treatment or a result of an innate preference to orient in that direction. The authors should have tested each subject 5 to 10 times before the mouse was presented to determine whether a position preference existed, and if found, run against the preference.

In a study of the responses of neonatal prairie rattlesnakes to odors of conspecific and heterospecific chemical cues, Scudder et al. (1988) used a Y-maze to assess the trailing behavior of eight neonates on conspecific odors. They ran two trials with each subject, a no trail control, and a trailing trial, where one arm of the maze (chosen randomly) was scented with a live conspecific. Whether or not the test subject chose the correct trail was recorded. The control trial was intended to test for non-random position preferences in the test population. They found a significant preference for the arm with the conspecific trail. Unfortunately, the test for position preferences was done

incorrectly. It is impossible to determine a position preference in a single trial. If only two subjects had turning biases, each in the opposite direction, the investigators would still have found random turning. During the trail trials, individuals could have been tested toward their bias, and the investigators would never know. If this occurred, the significant preference for a conspecific trail is not due to trailing, but to a turning bias.

Melcer and Chiszar (1989a) recognized the importance of using arbitrary stimuli in discrimination tasks. Consequently, they performed a series of seven experiments similar to experiments described in Melcer and Chiszar (1988) testing the utility of perfumes as discriminative stimuli. In experiment one, forty prairie rattlesnakes were counter balanced across four treatment conditions. There were ten subjects in each of the four treatment conditions: strike and perfume misted, no strike and perfume misted, strike and water misted, non-strike and water misted. In the strike trials, snakes were allowed to strike either a perfume or water misted carcass. In the non-strike trial, a perfume or water misted carcass was suspended in the home cage for five seconds. Snakes were then allowed to choose between a perfume and water misted carcass (both of these were not envenomated). The type of carcass ingested (perfume or water) was recorded. Of the twenty strike snakes, 16 consumed the same type of carcass as they struck. In the no-strike snakes, 11 failed to feed and the other nine chose randomly. The no strike snakes suggest there is no innate preference for the perfume or water misted carcasses. However, since both odors were not presented together during the initial five seconds of the experiment, it is unknown if the preference for the same carcass struck is a true preference or avoidance of the odor not previously experienced.

Experiment two extended these results to six additional species. Unfortunately, interspecific comparisons could not be made because of small sample sizes. Once again, interpretation is made more difficult because the data were pooled across species. The results were the same as in experiment one.

Experiment three tested the ability of 20 *C. viridis* to discriminate between carcasses misted with three different perfumes. Five snakes struck Jungle Gardenia misted prey and five struck Tea Rose misted prey. Then they were allowed to choose (ingest) between two non-envenomated carcasses, each misted with either of these two perfumes. An additional ten snakes struck and chose between Tea Rose and Halston misted carcasses (five struck each of the two perfume misted carcasses). The results showed that snakes could discriminate between two different perfume misted carcasses. However, this choice cannot be attributed to a learned preference because both carcasses were not present during the time of striking. It is possible that snakes were avoiding the unfamiliar odor, not choosing the familiar one. Additionally, multiple trials need to be run with each subject to implicate a learned discrimination.

In experiment four, snakes were allowed to strike misted carcasses as in experiments 1-3, but for the discrimination tests, carcasses were confined in mesh baskets similar to those used by Duvall et al. (1980). Preference was determined by counting the number of TF directed at each bag and the amount of time a snake's head was facing and within 2.5 cm of each bag. Five prairie rattlesnakes were tested twice. Each struck a perfume misted carcass once and a water misted carcass once. During the first two minutes of the trial, significantly more TF and time were directed toward the carcass type

that was struck. Again, discriminative stimuli were not presented simultaneously, and only one trial for each condition was carried out.

Experiment five determined how long the preference remained within individuals. Snakes were tested for their preference either 5 minutes or 60 minutes following a strike. Each snake was tested in each time condition, the eight subjects were counterbalanced so four received the 5-minute trial first and the other four received the 60-minute trial first. Snakes retained their preference for carcasses matching the one struck for at least 60 minutes following the strike.

Experiment six was identical to experiment five, except the prey choice test was 120 minutes following the strike. Snakes failed to show a preference for the matching carcass in this experiment, though the total number of investigatory TF's were equivalent to those in experiment five. Melcer and Chiszar concluded that the chemical search image of the snakes disappears between 60 and 120 minutes following the strike. The statistical power of this experiment is probably quite low given that the sample size was only eight individuals.

Experiment seven attempted to incorporate distinctive odors onto prey in a more natural way. Two populations of laboratory rodents were fed different diets. One diet was dusted with ground cinnamon and the other with cocoa. Experimental procedures were the same as in previous experiments. Snakes showed a preference, as indicated by time and TF, directed toward carcasses that matched the one that was struck. Recall, both carcasses were not envenomated. Hence rodents may acquire some chemical information from their diets. It was unknown if the rodents had traces of the spices in their fur or if the chemicals were incorporated into the rodent through ingestion. As in all of the

previous experiments summarized in this study, the discriminative stimuli were not presented simultaneously during the strike, so preference cannot be fully distinguished from avoidance of the odor not encountered.

Melcer and Chiszar (1989b) extended their previous (1988, 1989a) investigations by testing the possibility that mere exposure might induce a preference toward prey with matching chemical cues. In experiment one, six adult prairie rattlesnake were exposed to a perfume or water misted carcass ($n = 3$ in each treatment) for six seconds, but were not allowed to strike the carcass. A perfume and water misted carcass were each placed into mesh bags. The amount of time oriented toward and the number of TF were recorded for each type of prey. No preference was evident, though Melcer and Chiszar reported that two snakes did not investigate either carcass, two had numerical values favoring the matching carcass, and two had numerical values in the opposite direction. These results underscore the importance of individual data with multiple trials and larger sample sizes for improved statistical power.

The same six snakes from experiment one were tested in experiment two. This experiment was identical to experiment one, except the perfume concentration used was 100 times more concentrated and subjects were tested once in the perfume misted treatment and once in the water misted treatment. Ordering was counterbalanced. Again there was no preference, as measured by time or TF for the matching carcass.

Experiment three tested for the effects of exposure to perfume odors before a strike and effects of exposure to perfume odors after a strike. They also replicated the experiment, described above (Melcer and Chiszar, 1989a; experiment 3). Pre- and post-strike exposure to perfume misted mice did not affect the number of TF and time directed

toward matching and non-matching carcasses. Replication of experiment three from Melcer and Chiszar (1989a) was consistent with previous findings. Again, for a discrimination experiment, stimuli should be paired more than once, and discriminative stimuli should be presented simultaneously.

Experiment four replicated experiment three from Melcer and Chiszar (1989a) with the exception that the perfume was diluted by a factor of 100. There was a weak preference for the matching carcass, which would probably be stronger with a larger sample size. These investigations illustrate the importance of using novel discriminative stimuli.

Duvall et al. (1990) conducted four experiments, in which they investigated the propensity of prairie rattlesnakes to discriminate between cages containing clean versus soiled mouse shavings and cages containing live mice versus soiled shavings. Preference was measured by percent time spent near a given cage, time spent in ambush postures near a given cage, TF on a given side of the testing arena, mouth gapes toward a given cage, nudges, peers, and strikes directed at a cage during 3- to 10-minute intervals on three consecutive days. In experiment 1 there were no differences in any of the dependent variables directed toward either cage. In experiment 2, the same treatments were used but the odor sources were elevated as in experiment 1. The results indicated that more ambush postures were observed near cages with soiled bedding.

In experiment 3 with live mice versus soiled shavings, snakes spent more time near the live mice, in ambush postures near live mice and exhibited more peers directed toward live mice. Further, these responses increased over days. Males but not females decreased the number of nudges across trials.

Experiment 4 was designed as a semi-natural experiment in which snakes were placed in a large indoor enclosure with a dirt and gravel floor for 20 days. Cages with soiled and clean shavings were placed in an enclosure during days 15-20 and the position of the snakes in the enclosure recorded ten times per day for six days. Snakes spent significantly more time near the cages containing soiled shavings on days 15-17, but during days 18-20 snakes were not attracted to the cages containing soiled shavings.

While discrimination was evident in several of the experiments, the investigators suggest evolution has shaped the propensity of these snakes to adopt ambush postures near sites where mice may occur or where they have seen mice in the past, and that this propensity may vary between populations of *C. viridis*. However, they did not test the mechanism by which these discriminations were made. The test subjects were captured as adults; hence I do not know their history. Animals could have learned to associate specific stimuli with the probability of successful foraging. I recommend designing an experiment similar to Clark (2004c) in which adopting ambush postures in the presence of certain discriminative stimuli (odors or visual cues) and differentially reinforcing those postures

Chiszar et al. (1991) performed a typical SICS experiment (see above, Chiszar et al., 1982) to assess where on the prey, chemical cues perceived by the snake originate. It has been assumed these cues come from the rodent's integument (for example, see Melcer and Chiszar, 1988; 1989a,b). Twelve snakes were divided into two groups. Group A was allowed to strike a non-lubricated latex condom containing a euthanized weanling rat. Group B snakes were allowed to strike a non-lubricated latex condom containing cotton balls soaked in water. The number of TF per minute was recorded for

the following 30 minutes. While both groups of snakes exhibited SICS, Group A displayed significantly higher TF rates than group B snakes. It is noteworthy that the level of SICS by Group A was not as typical as SICS exhibited following a strike on an intact rodent. The investigators conclude the difference between group A and B is the result of internally derived chemical cues gathered by the fangs during a strike. However the mechanism of discrimination is not considered. The snakes used in the experiment were long-term captives and so could have learned about the internally derived cues prior to the experiment. I recommend multiple trials per individual and the use of novel chemical stimuli that are differentially reinforced. For example, two types of cotton filled condoms, each laced with a different chemical signal, could be presented and allowed to be struck, but only one would get reinforced with a successful feeding bout. If learning is involved, rates of TF should increase with experience in response to the positive discriminative stimulus.

It is possible that rattlesnakes have the ability to learn associations with novel stimuli and an innate capacity to recognize internal rodent chemicals. These may be separated by using ingestively naïve snakes in an experiment similar to the one described in the previous paragraph. Both novel chemicals and rodent cues should be used. If the ability to discriminate rodent odors is innate behavior should not change appreciably with experience.

Chiszar et al. (1999b) added *Crotalus atrox* to the list of species that discriminate envenomated from non-envenomated mice. Again the mechanism by which the snakes discriminated envenomated from non-envenomated prey was not indicated. An important contribution of this study was the preparation of a dose response curve. It was

determined that the minimum perceptible dose of venom by *Crotalus atrox* was between 6.0 and 7.1 mg.

The ability of rattlesnakes to “meter” or control the amount of venom delivered in a predatory strike is debated in the literature (Hayes et al., 2002; Young et al., 2002). Regardless, circumstantial evidence suggests rattlesnakes can modulate the amount of venom expended depending on the size of the target (Hayes et al., 2002). Hayes (1995) and Hayes et al. (1995) showed that *C. viridis* and *C. oreganus* expended more venom when striking large prey, relative to small prey, and that experience had an effect on the amount of venom injected (Hayes, 1995). That is, snakes discriminate between large and small prey, and make a differential response (venom mass) based on the discriminative stimulus/stimuli present during the strike. While this may be the case, the cues used and the mechanism by which discrimination is achieved is unknown. Hayes et al. (1995) suggests that visual/thermal cues are used. It has also been shown that the amount of venom injected is correlated with time to death of the prey (Hayes, 1995).

I recommend separating the discriminative cues and the consequence and re-pairing them in a factorial manner. By doing so, if snakes do meter, by making the perceived time to death longer (by withholding it from the snake) for small prey, a snake should proceed through successive trials by injecting more venom in to small prey so it “dies” faster. Obviously, all the relevant controls discussed previously for instrumental conditioning and discrimination experiments should be implemented.

The recent work of Clark (2004a) suggests that timber rattlesnakes are capable of discriminating between kin and non-kin. He placed snakes into 4 different types of pairs: female siblings, female non-siblings, male siblings, and male non-siblings. He measured

discrimination by recording the distance between individuals in the pair and the number of times individuals were in contact or entwined. He found that female siblings tended to rest closer to each other than individuals in other types of pairs, and they were found more often in contact or entwined. Male, non-siblings were never found entwined. Unfortunately, he did not identify the discriminative stimuli. Further, individuals were not given a simultaneous choice to rest near a sibling or non-sibling. At this time it is impossible to know if the apparent discrimination between kin and non-kin is innate or learned. His snakes were reared together until the first ecdysis, so imprinting is a possible explanation. However, phenotype matching (Holmes and Sherman, 1983) or some other similar rule may also explain the association among kin.

In another study by Clark (2004b), he investigated discrimination of potential prey by timber rattlesnakes. He tested responses to 8 prey extracts and found that captive raised snakes discriminated between small mammal extract and all other prey extracts. Discrimination was determined by the tongue flick ambush score, which is a composite based on the latency to assume an ambush posture plus the number of tongue flicks directed toward an extract trail. All animal extracts were tested against a tap-water blank. Clark did not speculate on how the discrimination among extracts was formed and it is unknown whether it is innate or learned. Since discriminative stimuli were not presented simultaneously (a blank control is not an appropriate discriminative stimulus) it is possible the small rodent extracts scored highest because they were most similar to the diet the snakes were fed in captivity. I recommend further experiments in which two different novel extracts serve as discriminative stimuli in several training trials in which one of them is paired with food. Then perform the discrimination experiment and

calculate tongue flick ambush scores to both stimuli. If the snakes can discriminate, the positive discriminative stimulus should receive a higher tongue flick ambush score.

In a third study by Clark (2004c), learning was implicated in ambush-site selection by timber rattlesnakes. In the first experiment, snakes were tested for their tongue flick ambush score to large or small rat extracts prior to feeding on a large or small rat. Extracts were prepared with methanol and presented on dampened paper towels with a methanol blank as a control. Tongue-flick ambush scores were recorded again following the opportunity to feed on a large or small rat. Snakes fed a large rat had an increased response to the extract following the feeding opportunity, but snakes fed a small rat did not show an increased response to the extract.

In the second experiment, woodchuck extract was daubed onto the fur of a large or small mouse and the snake's response was recorded before and after a feeding opportunity. Snakes fed a large mouse showed an increase response to the woodchuck odor following the feeding opportunity. Snakes fed the small mouse daubed with woodchuck scent did not increase their response.

In a third experiment, goldfish odor was daubed onto the fur of a large mouse and the responses to the extract were recorded before and after a feeding opportunity. The results indicated no differences between the two conditions. Again, pairing the discriminative stimulus with a blank control is a problem as discussed previously. Further, extract concentration may have differed between subjects in the woodchuck experiments because extracts were applied over the surface of large and small mice. Spreading the extract over a larger surface area increases the active space of the

chemicals being perceived by the snake, which could theoretically result in an increase in responsiveness.

Several papers I reviewed did not necessarily demonstrate learning, but possessed elements I think are important in the analysis of learning in rattlesnakes. Several studies of trailing and prey capture (Chiszar and Radcliffe, 1976; Chiszar, et al. 1981; Chiszar et al., 1986a,b; Cruz et al. 1987; Scudder et al., 1992) demonstrate the importance of species comparisons. While many of these studies found no interspecific differences (e.g. Scudder et al., 1992), this may be a result of small sample sizes and low statistical power. Nonetheless, a complete understanding of learning phenomena among rattlesnakes from a phylogenetic perspective will require studies utilizing many different rattlesnake species.

Trailing behavior (and the dependent variables associated with its quantification) may be useful for testing other issues associated with learning. For example, chemical trails are used for navigation, but can rattlesnakes use other cues (e.g., visual) in the environment to find their way (Landreth, 1973)? Additionally, Chiszar et al. (1981) demonstrated the important impact of disturbance and environment on trailing behavior. Knowledge of the effects of disturbance on learning would also be important. The “spook factor” described by Brown (1993, 2005) is a case in point. Brown observed that timber rattlesnakes that were captured at specific rock outcroppings and handled by human investigators for processing, failed to re-appear at those same outcroppings for several consecutive years before resuming basking activities at the site.

As I have discussed for habituation and classical conditioning, the research opportunities for instrumental and operant conditioning are wide open. For example, what is the effect of delayed reinforcement; are rattlesnakes sensitive to contingencies of

reinforcement; what is the behavior associated with probability learning; are rattlesnakes sensitive to avoidance and omission contingencies? In addition to generating data on fundamental issues in the study of learning, applied questions similar to those asked for classical conditioning can be explored.

Recommendations

In closing I will make several recommendations. The most obvious is that greater attention be paid to incorporating learning designs into rattlesnake research. In writing this review I was rather shocked on how little research there was investigating learning in this important animal group. Current research on discrimination in rattlesnakes can easily be incorporated into experimental designs to investigate learned phenomena in rattlesnakes. The technique of Melcer and Chiszar (1988) is especially useful. The work of Brown on the “spook factor” (1993, 2005) and the findings of Clark (2004a) on kin selection and ambush site selection deserve further investigation.

When designing experiments on learning, researchers must become familiar with the issues in the psychology of learning, particularly the comparative analysis of learning. Animals, for instance, should certainly receive more than one exposure to a stimulus and more than one “preference” trial. Moreover, combining data across species, considering subjects used in multiple experiments as independent, and failure to consider the influence of behavior that may have been learned during captive maintenance or the pre-training phase of an experiment is problematic for any learning interpretation. These issues have been addressed in this chapter. It is not enough for a researcher to slap together a response and consequence and consider that learning.

A particularly important issue is the use of control groups. It is difficult to attribute a particular rattlesnake finding to learning unless alternative explanations are ruled out. Studies of habituation, for example, must utilize a control for effector fatigue and sensory adaptation. The best way to do this is to incorporate a dishabituating stimulus. Moreover, initial studies of classical conditioning must employ a control group receiving the same number of CSs and USs but presented unpaired. The unpaired control group (or the use of a discrimination procedure when a within subject experimental design is employed) is necessary to assess the amount of pseudoconditioning. The use of a control group in which the response and consequence are unpaired is also necessary for studies of instrumental and operant conditioning.

I also recommend that researchers create a catalog of stimuli that can serve as positive and negative reinforcers, punishments, conditioned, unconditioned, and discriminative stimuli. Before a learning experiment can be designed, researchers must know what will motivate the rattlesnake and for how long. The search for positive reinforcers such as preferred foods is especially critical. In the absence of available positive reinforcers, aversive stimuli can be used but a comparative analysis of learning cannot rest solely on the use of aversive events.

The search for appropriate stimuli that can be used in learning experiments go hand in hand with the development of techniques to study training variables known to influence learning (i.e., CS-US intervals, magnitude of reward). For example, instead of developing mazes, I suggest development of automated classical and operant conditioning techniques that can generate quantitative data. Quantitative data can be used to compare similar data on other species and can be used to create a foundation of

reproducible data that can be used in qualitative research designs where the learning tasks involve more ecologically relevant learning tasks. These tasks could include predator avoidance, learned recognition of unpalatable food, learned recognition of palatable food, learned conspecific recognition, and habitat recognition. I would also suggest that examples of individual data be reported in learning studies.

The development of apparatus and the gathering of quantitative data must find an outlet. I encourage journal editors and reviewers to support manuscripts describing apparatus and to report quantitative data. Especially important is the support of manuscripts that report negative results. The reporting of negative results will give researchers an idea of what worked and what did not, saving valuable time.

In conclusion, I believe the study of learning in rattlesnakes is a wide-open area with many contributions to be made. I hope that this chapter will encourage scientists working on rattlesnake behavior to embark on a research program in which the psychology of learning forms a central part.

Literature Cited

- Abramson, C. I. 1994. A Primer of Invertebrate Learning. American Psychological Association, Washington, DC.
- 1997. Where have I heard it all before: Some neglected issues of invertebrate learning. *In* G. Greenberg and E. Tobach (eds.), Comparative Psychology of Invertebrates: The Field and Laboratory Study of Insect Behavior, pp. 55-78. Garland Publishing, New York.
- Abramson, C.I., P. M. Armstrong, R. A. Feinman, and R. D. Feinman. 1988. Signaled

- avoidance in the eye withdrawal reflex in the green crab. *Journal of the Experimental Analysis of Behavior* 50:483-492.
- Abramson, C. I., J. Squire, A. Sheridan, and P. G. Mulder, Jr. 2004. The effect of insecticides considered harmless to honey bees (*Apis mellifera* L.): Proboscis conditioning studies using the insect growth regulators Confirm[®]2F (Tebufenozide) and Dimilin[®]2L (Diflubenzuron). *Environmental Entomology* 33:378-388.
- Altamari, W. 1998. *Venomous Snakes: a Safety Guide for Reptile Keepers*. Society for the Study of Amphibians and Reptiles. Herpetological Circulars, No. 26.
- Amsel, A. 1989. *Behaviorism, Neobehaviorism, and Cognitivism in Learning Theory: Historical and Contemporary Perspectives*. LEA, Hillsdale, New Jersey.
- Amsel, A. and M. E. Rashotte. 1984. *Mechanisms of Adaptive Behavior: Clark L. Hull's Theoretical Papers, with Commentary*. Columbia University Press, New York.
- Bitterman, M. E. 1962. Techniques for the study of learning in animals: Analysis and classification. *Psychological Bulletin* 59:81-93.
- 1965. Phyletic differences in learning. *American Psychologist* 20:396-410.
- Bolles, R. C. 1970. Species-specific defensive reactions and avoidance learning. *Psychological Review* 77:32-48.
- Brown, W. S. 1993. *Biology, Status, and Management of the Timber Rattlesnake (Crotalus horridus): A Guide for Conservation*. Society for the Study of Amphibians and Reptiles Herpetological Circulars No. 22.
- 2005. Talk presented at 3rd Annual Timber Rattlesnake Symposium of the Southern Tier. April, 2005.

- Burghardt, G. M. 1977. Learning processes in reptiles. *In* C. Gans & D. Tinkle (eds.), *The Biology of the Reptilia*. Vol. 7. pp. 555-681. Academic Press, New York.
- Byrne, J. H. 2003. *Learning & memory* 2nd ed. MacMillan, New York.
- Campbell, J. A., D. R. Formanowicz, Jr., and E. D. Brodie, Jr. 1989. Potential impacts of rattlesnake roundups on natural populations. *Texas Journal of Science* 41:301-317
- Clark, R. W. 2004a. Kin recognition in rattlesnakes. *Proceedings of the Royal Society of London Series B-Biological Sciences (Suppl.)* 271:S243-245.
- 2004b. Timber rattlesnakes (*Crotalus horridus*) use chemical cues to select ambush sites. *Journal of Chemical Ecology* 30:607-617.
- 2004c. Feeding experience modifies the assessment of ambush sites by the timber rattlesnake, a sit-and-wait predator. *Ethology* 110:471-483.
- Chiszar, D., and C. W. Radcliffe. 1976. Rate of tongue flicking by rattlesnakes during successive stages of feeding on rodent prey. *Bulletin of the Psychonomic Society* 7:485-486.
- Chiszar, D., K. Scudder, and L. Knight. 1976. Rate of tongue flicking by garter snakes (*Thamnophis radix haydeni*) and rattlesnakes (*Crotalus v. viridis*, *Sistrurus catenatus tergeminus*, and *Sistrurus catenatus edwardsi*). *Behavioral Biology* 18:273-283.
- Chiszar, D., K. Scudder, L. Knight, and H. M. Smith. 1978. Exploratory behavior in prairie rattlesnakes (*Crotalus viridis*) and water moccasins (*Agkistrodon piscivorus*). *Psychological Record* 28:363-368.

- Chiszar, D., C. W. Radcliffe, B. O'Connell, and H. M. Smith. 1981. Strike-induced chemosensory searching in rattlesnakes (*Crotalus viridis*) as a function of disturbance prior to presentation of rodent prey. *Psychological Record* 31:57-62.
- Chiszar, D., C. Andren, G. Nilson, B. O'Connell, J. S. Mestas Jr., H. M. Smith, and C. W. Radcliffe. 1982. Strike-induced chemosensory searching in old world vipers and new world pit vipers. *Animal Learning and Behavior* 10:121-125.
- Chiszar, D., B. O'Connell, R. Greenlee, B. Dementer, T. Walsh, J. Chiszar, K. Moran, and H. M. Smith. 1985. Duration of strike-induced chemosensory searching in long-term captive rattlesnakes at National Zoo, Audubon Zoo, and San Diego Zoo. *Zoo Biology* 4:291-294.
- Chiszar, D., C. W. Radcliffe, T. Byers, and R. Stoops. 1986a. Prey capture behavior of nine species of venomous snakes. *Psychological Record* 36:433-438.
- Chiszar, D., C. Radcliffe, and F. Feiler. 1986b. Trailing behavior of banded rock rattlesnakes (*Crotalus lepidus klauberi*) and prairie rattlesnakes (*Crotalus viridis viridis*). *Journal of Comparative Psychology* 100:368-371.
- Chiszar, D., G. Hobika, H. M. Smith, and J. Vidaurri. 1991. Envenomation and acquisition of chemical information by prairie rattlesnakes. *Prairie Naturalist* 23:69-72.
- Chiszar, D., K. DeWilde, M. Garcia, D. Payne, and H. M. Smith. 1999a. Strike induces chemosensory searching (SICS) in northern pacific rattlesnakes (*Crotalus viridis oregonus*, Holbrook, 1840) rescued from substandard husbandry conditions. II. Complete recovery after two years. *Zoo Biology* 18:141-146.

Chiszar, D., A. Walters, J. Urbaniak, H. M. Smith, and S. P. Mackessy. 1999b.

Discrimination between envenomated and non-envenomated prey by western diamondback rattlesnakes (*Crotalus atrox*): Chemosensory consequences of venom. *Copeia* 1999:640-648.

Coleman, S. R., A. Fanelli, and S. Gedeon. 2000. Psychology of the Scientist: LXXII.

Coverage of classical conditioning in textbooks in the psychology of learning, 1952-1995. *Psychological Reports* 86:1011-1027.

Couvillon, P. A. and M. E. Bitterman 1984. The overlearning-extinction effect and

successive negative contrast in honeybees (*Apis mellifera*). *Journal of Comparative Psychology* 98:100-109.

Cruz, E., S. Gibson, K. Kandler, G. Sanchez, and D. Chiszar. 1987. Strike-induced

chemosensory searching in rattlesnakes: a rodent specialist (*Crotalus viridis*) differs from a lizard specialist (*Crotalus pricei*). *Bulletin of the Psychonomic Society* 25:136-138.

Duvall, D., D. Chiszar, and J. Trupiano. 1978. Preference for envenomated rodent prey

by rattlesnakes. *Bulletin of the Psychonomic Society* 11:7-8.

Duvall, D, K. M. Scudder, and D. Chiszar. 1980. Rattlesnake predatory behavior:

mediation of prey discrimination and release of swallowing by cues arising from envenomated mice. *Animal Behaviour* 28:674-683.

Duvall, D., D. Chiszar W. K. Hayes, J. K. Leonhardt, and M. J. Goode. 1990. Chemical

and behavioral ecology of foraging prairie rattlesnakes. *Journal of Chemical Ecology* 16:87-101.

- Dyal, J. A. and W. C. Corning. 1973. Invertebrate learning and behavioral taxonomies. *In* W. C. Corning, J. A. Dyal, & A. O. D. Willows (eds.), *Invertebrate Learning: Vol. 1. Protozoans through Annelids*, pp. 1-48. Plenum, New York.
- Gans, C. 1966. The biting behavior of solenoglyph snakes-its bearing on the pattern of envenomation. *Proceedings of the International Symposium on Venomous Animals*. Instituto Butantan, Sao Paulo, Brazil.
- Gillingham, J. C., C. C. Carpenter, and J. B. Murphy. 1983. Courtship, male combat, and dominance in the western diamondback rattlesnake, *Crotalus atrox*. *Journal of Herpetology* 17: 265-270.
- Gormezano, I. 1984. The study of associative learning with CS-CR paradigms. *In* D. L. Alkon and J. Farley (eds.), *Primary Neural Substrates of Learning and Behavioral Change*, pp. 5-24. Cambridge University Press, Cambridge.
- Gormezano, I. and E. J. Kehoe. 1975. Classical conditioning: Some methodological-conceptual issues. *In* W. K. Estes (ed.), *Handbook of Learning and Cognitive Processes*, Vol. 2. *Conditioning and Behavior Theory*, pp. 143-179. LEA, Hillsdale, New Jersey.
- Gormezano, I., E. J. Kehoe, and B. S. Marshall. (1983). Twenty years of classical conditioning research with the rabbit. *In* J. M. Sprague and A. N. Epstein (eds.), *Progress in Psychobiology and Physiological Psychology*, Vol. 10, pp. 197-275. Academic Press, New York.
- Greene, H. W. 2003. Appreciating rattlesnakes. *Wild Earth* 13: 28-32.

- Halpern, M. 1992. Nasal chemical senses in reptiles: structure and function. *In* C. Gans, and D. Crews (eds.), *Biology of the Reptilia*, Vol. 18, pp. 423-523. Univ. Chicago Press, Chicago.
- Hampton, R. and J.C. Gillingham. 1989. Habituation of the alarm reaction in neonatal garter snakes, *Thamnophis sirtalis*. *Journal of Herpetology* 23:433-434.
- Harris, J. D. 1943. Habituation response decrement in intact organisms. *Psychological Bulletin* 40:385-422.
- Hayes, W. K. 1995. Venom metering in juvenile prairie rattlesnakes, *Crotalus viridis viridis*: effects of prey size and experience. *Animal Behaviour* 50:33-44.
- Hayes, W. K., P. Lavin-Murcio, and K. V. Kardong. 1995. Northern pacific rattlesnakes (*Crotalus viridis oreganus*) meter venom when feeding on prey of different sizes. *Copeia* 1995:337-343.
- Hayes, W. K., S. S. Herbert, G. C. Rehling, and J. F. Gennaro. 2002. Factors that influence venom expenditure in viperids and other snake species during predatory strikes. *In* G. W. Schuett, M. Hoggren, M. E. Douglas, and H. W. Greene (eds.), *Biology of the Vipers*, pp. 207-234. Eagle Mountain Publishing, Eagle Mountain, Utah.
- Hirsch, J. and M. Holliday. 1988. A fundamental distinction in the analysis and interpretation of behavior. *Journal of Comparative Psychology* 102:372-377.
- Holmes, W. G., and P. W. Sherman. 1983. Kin recognition in animals. *American Scientist* 71:46-55.

- Kandler, K, and D. Chiszar. 1986. Spatial orientation by prairie rattlesnakes (*Crotalus viridis*) following the predatory strike. *Bulletin of the Psychonomic Society* 24:169-170.
- Kleinginna, Jr., P. R. 1970. Operant conditioning in the indigo snake. *Psychonomic Science* 18:53-55.
- Landreth, H. F. 1973. Orientation and behavior of the rattlesnake, *Crotalus atrox*. *Copeia* 1973:26-31.
- Lee, V. L. 1988. *Beyond Behaviorism*. LEA, New Jersey.
- Lubow, R. E. 1973. Latent inhibition. *Psychological Bulletin* 79:398-407.
- Mackintosh, N. J. 1974. *The Psychology of Animal Learning*. Academic Press, New York.
- Marmie, W., S. Kuhn, and D. Chiszar. 1990. Behavior of captive-raised rattlesnakes (*Crotalus enyo*) as a function of rearing conditions, *Zoo Biology* 9:241-246
- Melcer, T., K. Kandler, and D. Chiszar. 1988. Effects of novel chemical cues on predatory responses of rodent-specializing rattlesnakes. *Bulletin of the Psychonomic Society* 26:580-582.
- Melcer, T., and D. Chiszar. 1989a. Striking prey creates a specific chemical search image in rattlesnakes. *Animal Behaviour* 37:477-486.
- and ----- 1989b. Strike-induced chemical preferences in prairie rattlesnakes (*Crotalus viridis*). *Animal Learning and Behavior* 17:368-372.
- Mills, K. C. 1970. Attempts at escape and avoidance training with the rattlesnake using infrared light as the discriminative stimulus. Unpubl. Ph.D. dissertation., Claremont Graduate University.

- Morgan, C. L. 1894. Introduction to Comparative Psychology. London: Walter Scott.
- Reprinted in D. N. Robinson (ed.), 1977. Significant Contributions to the History of Psychology: Series D, Vol. 2, C. L. Morgan, pp. 1-382. University Publications of America, Washington.
- Murphy, R. W., J. Fu, A. Lathrop, J. V. Feltham, and V. Kovac. 2002. Phylogeny of the rattlesnakes (*Crotalus* and *Sistrurus*) inferred from sequences of five mitochondrial DNA genes. In G. W. Schuett, M. Hoggren, M. E. Douglas, and H. W. Greene (eds.), Biology of the Vipers, pp.69-92. Eagle Mountain Publishing, Eagle Mountain, Utah.
- Place, A. J., C. I. Abramson, and V. Ramirez. (in press) The rattlebox: an apparatus for studying learning in rattlesnakes. Herpetological Review.
- Radcliffe, C. W., D. Chiszar, and B. O'Connell. 1980. Effects of prey size on poststrike behavior in rattlesnakes (*Crotalus durissus*, *C. enyo*, and *C. viridis*). Bulletin of the Psychonomic Society 16:449-450.
- Razran, G. 1971. Mind in Evolution: An East-West Synthesis of Learned Behavior and Cognition. Houghton Mifflin, Boston.
- Scudder, K. M., and D. Chiszar. 1977. Effects of six visual stimulus conditions on defensive and exploratory behavior in two species of rattlesnakes. Psychological Record. 3:519-526.
- Scudder, K. M., D. Chiszar, H. M. Smith, and T. Melcer. 1988. Response of neonatal prairie rattlesnakes (*Crotalus viridis*) to conspecific and heterospecific chemical cues. Psychological Record 38:459-471.

- Scudder, K. M., D. Chiszar, and H. M. Smith. 1992. Strike-induced chemosensory searching and trailing behavior in neonatal rattlesnakes. *Animal Behaviour* 44:574-576.
- Seigel, S., and N. J. Castellan, Jr. 1988. *Nonparametric Statistics for the Behavioral Sciences*. 2nd ed. McGraw-Hill, New York.
- Sheldon, J. P. 2002. Operant conditioning concepts in introductory psychology textbooks and their companion web sites. *Teaching of Psychology* 29:281 -285.
- Skinner, B. F. 1989. *Recent Issues in the Analysis of Behavior*. Merrill, Columbus, Ohio.
- Suboski, M. D. 1992. Releaser-induced recognition learning by amphibians and reptiles. *Animal Learning and Behavior* 20:63-82.
- Teyler, T. J. 1984. *A Primer of Psychobiology: Brain and Behavior* 2nd ed. W. H. Freeman, New York.
- Thompson, R. F., and W. A. Spencer. 1966. A model phenomenon for the study of neuronal substrates of behavior. *Psychological Review* 73:16-43.
- Thorndike, E. L. 1949. *Selected Writings from a Connectionist's Psychology*. Appleton-Century-Crofts, New York.
- Tolman, E. C. 1932. *Purposive Behavior in Animals and Men*. University of California Press, Berkeley.
- Tulving, E. 1985. On the classification problem in learning and memory. *In* L. Nilsson and T Archer (eds.), *Perspectives on Learning and memory*, pp. 67-94. LEA, Hillsdale, New Jersey.
- Wickens, D. D., and C. D. Wickens. 1942. Some factors related to pseudoconditioning. *Journal of Experimental Psychology* 31:518-526.

- Winer, B. J., D. R. Brown, and K. M. Michels. 1991. Statistical Principles in Experimental Design. 3rd ed. McGraw-Hill, New York.
- Woods, P. J. 1974. A taxonomy of instrumental conditioning. *American Psychologist* 29:584-596.
- Young B. A., C. E. Lee, and K. M. Daley. 2002. Do snakes meter venom? *Bioscience* 12:1121-1126.
- Zimbardo, P. G. 1992. Psychology and Life. 13th ed. Harper Collins, New York.

CHAPTER II

FREQUENCY OF RATTLE USE BY FREE-RANGING WESTERN DIAMONDBACK RATTLESNAKES

The rattle system of rattlesnakes is an adaptive suite (Bartholomew, 1972) of morphological, physiological, and behavioral characters. Morphologically, the rattle is composed of several interlocking segments of retained exuviae (Klauber, 1972). Segments are formed over the style, which is itself the result of fusion of 8-10 caudal vertebrae (Zimmerman and Pope, 1948). The style is associated with three pairs of specialized shaker muscles that have high densities of mitochondria and sarcoplasmic reticula, and dense capillary beds (Schultz et al., 1980). Physiologically, the shaker muscles are among the most athletic muscles of any living organism, rivaling hummingbird flight muscle in this respect (Schaeffer et al., 1996). Behaviorally, the rattle is used in defense (Greene, 1988). The precise function is unknown, but it likely is as an aposematic or deimatic auditory display to potential predators (Greene, 1988, 1992; Fenton and Licht, 1990). These aposematic and deimatic functions probably differ with different predators (Fenton and Licht, 1990).

The evolutionary origin of the rattle and the associated physiology and morphology are unknown. Greene (1992) and Garman (1888) noted the striking qualitative similarity between the rattle button of rattlesnakes and the emergent tail spine of several species of new world crotalines. Moon (2001) described the apparent

intermediate condition and possible transition of the shaker muscles from non-tail vibrating colubrids through tail vibrating colubrids, and non-rattlesnake crotalids. Tail vibrating behavior is a common defensive behavior in many crotaline species (Greene, 1992), but is also demonstrated by many colubrids (Greene, 1973).

The rattle probably arose in a diminutive pit viper on the ~~Mar~~ an Occidental (Place and Abramson, 2004; Greene, 1998; Klauber, 1972) 20-30 million years ago (Knight et al., 1993). The relative size of the rattle is similar in most rattlesnake species (Cook et al., 1994). However, the pigmy rattlesnake (*Sistrurus miliarius*) has a significantly smaller rattle compared to other rattlesnakes (Cook et al., 1994) and loses the rattle to breakage more often than other species (Rowe et al., 2002). It is unclear if the small size of the rattle in *S. miliarius* is the ancestral condition or a derived state (Rowe et al., 2002).

A more complete understanding of the current function and the historical origin of the rattle requires an understanding of the context and frequency in which the rattle is used by free-ranging rattlesnakes. Several field experiments have assessed how multiple variables influence rattle use. These studies differed in approach. For example, Kissner et al. (1997) collected snakes in the field and tested all individuals at one location in a uniform fashion. Other studies used radio telemetry to test snakes in a systematic fashion, *in situ*, without disturbing the snakes prior to observing them (Duvall et al., 1985; Goode and Duvall, 1989; Graves, 1989; Prior and Weatherhead, 1994). Rowe et al. (2002) employed standardized methods (upon detection, each snake was approached and tapped on the snout with a gloved hand) to randomly encountered pigmy rattlesnakes. Comparisons between studies are difficult because of procedural differences. Moreover,

little emphasis has been placed on the actual frequency in which the rattle is employed in defensive encounters. My purpose was to 1) describe the frequency of rattle use in western diamondback rattlesnakes (*Crotalus atrox*), 2) assess differences in response probability between free-ranging, radio-tagged, and randomly encountered snakes, and 3) assess the current utility of the rattlesnake rattle.

Methods

Study organism.—*Crotalus atrox* is one of the largest species of rattlesnakes (typically 76-213 cm; Conant and Collins, 1998). In the study population, snakes are visible on the surface during every month of the year, but peak activity is concentrated during the spring (March-June) and fall (September-October; Ruthven and Kazmeier, unpubl. data). Common prey species on the study site for adult snakes are southern plains woodrat (*Neotoma micropus*), Ord's kangaroo rat (*Dipodomys ordi*), black tailed jackrabbit (*Lepus californicus*), and desert cottontail (*Sylvilagus auduboni*). Juvenile snakes probably consume juvenile rodents and lagomorphs, as well as Texas horned lizards (*Phrynosoma cornutum*), whiptail lizards (*Cnemidophorus gularis*), and fence lizards (*Sceloporus olivaceus* and *S. undulatus*).

Mammalian predators observed on the study area include badger (*Taxidea taxus*), coyote (*Canus latrans*), bobcat (*Felis rufus*), opossum (*Didelphis marsupialis*), and raccoon (*Procyon lotor*). Avian predators include Harris's hawk (*Parabuteo unicinctus*), crested caracara (*Polyborus plancus*), and greater roadrunner (*Geococcyx californicus*). Reptilian predators include Texas indigo snake (*Drymarchon corais erebennus*), coachwhip (*Masticophis flagellum*), Mexican milksnake (*Lampropeltis triangulum annulata*) and desert kingsnake (*Lampropeltis getula splendida*).

Study area.—The Chaparral Wildlife Management Area (28° 20' N, 99° 25' W) lies in southwestern Texas in DeMitt and LaSalle counties. Climate is characterized by hot summers and mild winters with an average daily minimum (January) temperature of 5° C, an average daily maximum (July) temperature of 37° C, a growing season of 249 to 365 days, and average annual precipitation (1951-1978) of 55 cm (Stevens & Arriaga, 1985). Average annual precipitation on the study site (1989-1999) was 54 cm (Chaparral Wildlife Management Area, unpubl. data). Precipitation patterns are bimodal with peaks occurring in late spring (May to June) and early fall (September to October). Short-term periods of drought are common and rainfall can be highly variable from one location to another (Norwine & Bingham, 1985). Topography is nearly level to gently sloping and elevation ranges between 168 and 180 m.

Domestic livestock have grazed the study area since the 18th century (Lehmann 1969). Cattle have been the major species of livestock since about 1870, whereas sheep were dominant from about 1750 to 1870. Before 1969, cattle grazing was continuous. From 1969 to 1984 cattle (cow/calf) grazed the area year-long, utilizing a four-pasture rest-rotation system. Cattle were absent from the area from 1984 to 1989. Cattle grazing resumed in 1990 and continued through 1999 utilizing a high intensity, low frequency grazing system in which cattle (stocker) rotated once through the study area during the period October through April. Stocking rates were considered low to moderate and averaged 1 Animal Unit (1 cow plus 1 calf) per 12 ha (D. Ruthven, personal communication).

Procedures.—Data were collected for randomly encountered snakes from May through August in 2001 and 2002, by visually searching for snakes on foot or by road

cruising at dusk and dawn. When snakes were found by road cruising, the vehicle was stopped a minimum of 4 m from the snake on the roadway and the snake was approached on foot. For the analysis, I did not separate snakes encountered by the two methods. Snakes found by road cruising were usually crossing paved or dirt roads within the confines of the management area where public access was restricted.

Seven adult *Crotalus atrox* (4♂:3♀) were relocated daily as part of another study investigating habitat use and movement patterns. Radio transmitters (Holohil Systems Ltd., Ontario, Canada and L. L Electronics, Mahomet, Illinois, USA) were surgically implanted in the coelomic cavity following the procedures of Hardy and Greene (1999, 2000). Data were collected only when snakes were visible.

Five variables were recorded for both the random encounter and radio-tagged snakes: sex, mass, snout-vent length (SVL), position (moving or coiled), and occurrence of rattling. These are known to influence rattling behavior (Kissner et al., 1997; Prior and Weatherhead, 1994; Duvall et al., 1985). Body condition was estimated by taking the residual from a regression of body mass on SVL (Secor and Nagy, 2005). I recognize that using residuals as an estimate for body condition is controversial. However, Secor and Nagy (2005) showed, using dual-energy X-ray absorption, that the residuals of body mass and SVL of diamondback water snakes (*Nerodia rhombifera*) are a significant predictor of body fat mass. It is important to note, however, that the snakes used by Secor and Nagy (2005) were post-absorptive. I could not assure that all the snakes I measured were post-absorptive. Nonetheless, I considered the residuals to be a rough indicator of body condition.

I did not record snake body temperature or ambient temperature during the behavioral observations. Temperature does not affect rattle responses in male and non-gravid female prairie rattlesnakes (*Crotalus viridis*; Kissner et al., 1997; Goode and Duvall, 1989). However, warm massasauga (*Sistrurus catenatus*) are more likely to rattle than cool ones (Prior and Weatherhead, 1994).

Statistical analysis.—Relationships between continuous variables and the occurrence of rattling were analyzed using logistic regression (Agresti, 1996). Independence among categorical variables was analyzed using the G^2 statistic for contingency tables and assessing standardized cell residuals (Agresti, 1996). Repeated observations made on radio-tagged snakes were analyzed using Cochran's Q test (Siegel and Castellan, 1988).

Results

Random encounters.—Forty-five snakes (23 ♂:10 ♀:12 unknown) were encountered and included in the analysis. Twenty-five (55.6%) rattled when approached. Thirty-two (71.1%) snakes were moving when first encountered. There was a marginally significant association ($G^2_1 = 3.55$, $P = 0.06$) between a snake's position and rattling. Coiled snakes were more likely to rattle than moving snakes. The propensity to rattle was not associated with sex ($G^2_2 = 1.13$, $P = 0.568$).

Logistic regression analysis of propensity to rattle and SVL was not significant ($\beta = 0.0$, $z = 0.017$, $df = 1$, $P = 0.897$). Neither mass nor body condition was a significant predictor of a rattle response (respectively: $\beta = 0.0$, $z = 0.001$, $df = 1$, $P = 0.969$; $\beta = 0.0$, $z = 0.002$, $df = 1$, $P = 0.961$). Logistic regression with small sample sizes may suffer from low statistical power (Agresti, 1996), so I confirmed the results of my logistic

regression analyses by performing 2-sample t-tests, with the two populations being those individuals that rattled and those that did not. Dependent variables for the t-tests were SVL, mass, and body condition. Snout-vent length, mass, and body condition did not differ significantly between snakes that rattled and snakes that did not (respectively: $T_{33} = 0.126$, $P = 0.901$; $T_{32} = 0.517$, $P = 0.970$; $T_{32} = 0.453$, $P = 0.963$).

Radio-tagged snakes.—One hundred and forty-four observations were made on the 7 radio-tagged snakes. Five encounters (3.5%) resulted in a rattle response. Twenty-five observations (17.4%) were made when a snake was moving. Making multiple observations on each individual violated the assumption of independence among data points, so Chi-square tests could not be performed. Five individual snakes each rattled one time. Two snakes never rattled in my presence. Five of the rattle responses were made when each snake was moving. One rattle response occurred when the individual was in a coiled position.

I applied Cochran's-Q test to the first ten observations for each radio-tagged snake and found no difference ($Q = 6.00$, $N = 7$, $df = 9$, $P = 0.740$) among the ten initial observations, but propensity to rattle was already low. This suggests the possibility of habituation to humans before my observations began.

Discussion

Western diamondback rattlesnakes have a notorious reputation for being aggressive and quick to rattle (Klauber, 1972). In my investigation, I found that 55.6% of snakes randomly encountered used their rattle. Snakes that were in a coiled position more commonly displayed the rattle response. This may be explained by relative risks of predation. Snakes rattling in the open habitats may attract attention in situations where

they are less able to escape once they are detected. Snakes rattling when coiled in a concealed position (e.g., under a shrub) may also attract attention, but they may be at a lower risk of attack because they are less accessible to the predator.

Alternatively, the propensity of snakes to rattle from a coiled position may represent a sampling bias. Snakes that rattle from a coiled position are much more likely to be discovered than snakes that remain silent in a coiled position. The data collected on radio-tagged snakes suggests that snakes tend to remain coiled more than they move. And coiled snakes do not rattle very often.

Interestingly, in snakes that I observed repeatedly, only 3.5% of my observations were of rattling snakes. These results suggest repeated observations on a limited number of individuals may bias assessment of rattle use. Prior and Weatherhead (1994) estimated the frequency of rattle use in radio-tracked massasauga (*Sistrurus catenatus*) being approached by human observers. Less than half (34%-42%) of their observations were of snakes that rattled, a notably higher frequency than I observed. This may be attributable to species differences in propensity to rattle. Alternatively, the snakes in my study could have been showing long-term habituation (Carew, et al., 1972) to the presence of humans. All of the radio-tagged snakes were captured as adults, so their history is unknown. The snakes observed in my study were part of another study investigating habitat use and movement patterns. In addition to the behavioral data presented here several habitat variables were also recorded on each observation. On average, I estimate data collection took about 5 to 8 minutes each day and the observer was usually less than two meters from the snake while recording data. Additionally, hunters routinely use the wildlife area for a large part of the year.

Prior and Weatherhead (1994) found no evidence of habituation in their population, although the repeated measurements were not provided. Laboratory investigations of short and long-term habituation (see chapter 4) show that both types of habituation do occur, but there is tremendous inter-individual variation in the rate and trajectory of habituation curves within and between days.

Most of the radio-tagged snakes rattled when they were moving. Again, the radio-tagged snakes were adults with unknown histories. These snakes may have had several encounters with humans prior to my study and learned to perform specific behaviors in the presence of humans. For example, snakes often go unnoticed when they are coiled beneath a shrub, hence not rattling is reinforced. When the same snakes are moving about their environment and encounter a human, rattling behavior results in the human avoiding the snake, thus reinforcing rattling behavior. These two situations are examples of instrumental or operant conditioning. Further laboratory-based approaches need to be undertaken to better understand the contingencies involved in these processes.

Pigmy rattlesnakes (*Sistrurus miliarius*) are more likely to exhibit an active behavioral response (i.e., strike or flee) in the presence of a predator when they are moving (Glaudas et al., 2005). If rattling is considered an active response, my finding in random encounter snakes is in accordance with those of Glaudas et al. (2005). Unfortunately, their population of rattlesnakes rattled too infrequently (rattle responses reported in Rowe et al., 2002) for comparison with my present study.

I found no sex related effects associated with rattle use in *C. atrox*. These results are similar to those found for *C. viridis* (Goode and Duvall, 1989, Kissner et al., 1997). I did not observe any gravid females, so it is still unknown if gravid female *C. atrox*

exhibit differences in behavior related to reproductive condition similar to *C. viridis* (Goode and Duvall, 1989, Kissner et al., 1997).

Table 2.1 shows the variation in frequency of rattle use both within and between species of rattlesnakes. There is no apparent relationship between rattle use and species. For example, larger species (e.g., *C. atrox*), do not rattle more often than smaller species (e.g. *S. catenatus*), or vice versa. As Rowe et al. (2002) suggest for *Sistrurus miliarius*, propensity of rattle use may be related to predation pressure. Species (or populations) that have historically experienced limited predation may utilize their rattle less frequently.

Alternatively, the tendency of an individual to use its rattle during an encounter with a predator may be dependent on an internally based, behavioral threshold. The behavioral threshold to rattle varies within and between individuals and depends on evolutionary history as well as numerous environmental and subject variables, such as ambient temperature, distance to cover, social context, type of predator, body temperature, body condition, and reproductive condition. If the behavioral threshold for rattling is high, an individual will not rattle or will delay rattling until sufficiently provoked. If the threshold for rattling is low, an individual will be quick to rattle. This behavioral homeostasis model (Eisenstein et al., 2001) can be tested empirically in the laboratory. While behavioral thresholds cannot be measured directly because they are internal states, they are outwardly expressed as overt behavior. I recommend simple laboratory experiments in which rattlesnakes are repeatedly stimulated to rattle and components of the rattle response are quantified over several trials. Snakes with a high

behavioral threshold to rattle should show longer initial latencies to rattle and individual rattling bouts should be shorter than in snakes with low thresholds.

Most published observations on rattle use suggest that rattlesnakes generally use the rattle in approximately 50 percent of their encounters with predators (Table 2.1). It is unknown if rattling behavior is currently subject to natural selection. However, if the rattle and rattling behavior are adaptations, they should correlate significantly with fitness (Endler, 1986). Although I did not measure fitness directly, I did calculate body condition, a potentially useful proxy for fitness (Marshall et al., 1999). I found no significant relationship between body condition and rattle use. Rowe et al. (2002) found that rattle-chain length was not significantly correlated with growth rates, survival, nor male and female reproductive success in pygmy rattlesnakes. While *S. miliarius* may be a special case among rattlesnakes, the data I present on rattle use suggests that rattling behavior similarly does not influence fitness. Additional work needs to be done to more precisely quantify fitness consequences of rattle morphology and rattle use in a number of rattlesnake taxa.

I recommend that rattlesnake biologists continue to gather natural history data related to rattle use. As Greene (1986) so stressed, natural history data-sets have important implications for evolutionary studies. I strongly urge publication of rattle use data in venues such as *Herpetological Review* and regional natural history journals such as *The Southwestern Naturalist*. Once these data become more readily available we will be able to better assess the current utility and historical origin of the unique, crotaline rattle.

Table 2.1 Summary of interspecific differences in rattle use among species of rattlesnakes. The numbers in parentheses next to proportion of use are the sample sizes. For the type of study, R = random encounters, T = radio tagged, and E = field or laboratory experiment.

Species	Proportion of use	Type of study	Source
<i>Sistrurus catenatus</i>	32%-42% (1740)	T	Prior and
<i>catenatus</i>			Weatherhead, 1994
<i>S. c. catenatus</i>	36% (372)	T	Hedgecock, 1992
<i>S. miliarius</i>	3% (1,350)	R	Rowe et al., 2002
<i>barbouri</i>			
<i>Crotalus atrox</i>	42% (24)	E	Young and Aguiar, 2002
<i>C. atrox</i>	56% (45)	R	Current study
<i>C. atrox</i>	4% (144)	T	Current study
<i>C. helleri</i>	53% (55)	R	Sweet, 1985
<i>C. oreganus</i>	97% (27)	R	Sweet, 1985
<i>C. viridis</i>	83% (89)	E	Kissner et al., 1997
<i>C. viridis</i>	100% (5)	T	Graves, 1989

LITERATURE CITED

- Agresti, A. 1996. An Introduction to Categorical Data Analysis. John Wiley and Sons, New York.
- Bartholomew, G. A. 1972. Body temperature and energy metabolism, *In: Animal physiology: Principles and adaptations*. M. S. Gordon (ed.), (pp. 298-368) Macmillan, New York.
- Blair, W. F. 1950. The biotic provinces of Texas. *Tex. J. Sci.*, 2:93-117.
- Carew, T. J., H. M. Pinsker, and E. R. Kandel. 1972. Long-term habituation of a defensive withdrawal reflex in *Aplysia* *Science* 175:451 -454.
- Conant, R., and J. T. Collins. 1998. Reptiles and Amphibians: Eastern/Central North America. 3rd Ed. Houghton-Mifflin, Boston, MA.
- Cook, P. M., M. P. Rowe, and R. W. Van Devender. 1994. Allometric scaling and interspecific differences in the rattling sounds of rattlesnakes. *Herpetologica* 50:358-368.
- Duvall, D., M. B. King, and K. J. Gutzwiller. 1985. Behavioral ecology and ethology of the prairie rattlesnake. *National Geographic Research* 1:80-112.
- Eisenstein, E. M., D. Eisenstein, and J. C. Smith. 2001. The evolutionary significance of habituation and sensitization across phylogeny: a behavioral homeostasis model. *Integrative Physiological and Behavioral Science* 36:251-265.
- Endler, J. A. 1986. Natural Selection in the Wild. Princeton University Press, Princeton, New Jersey.

- Fenton, M. F., and L. E. Licht. 1990. Why rattle snake? *Journal of Herpetology* 24:274-279.
- Garman, S. 1888. The rattle of the rattlesnake. *Bulletin of the Museum of Comparative Zoology*. 13:259-268.
- Glaudas, Z., T. M. Farrell, and P. G. May. 2005. Defensive behavior of free-ranging pygmy rattlesnakes (*Sistrurus miliarius*). *Copeia* 2005:196-200.
- Goode, M. J., and D. Duvall. 1989. Body Temperature and defensive behavior of free-ranging prairie rattlesnakes, *Crotalus viridis viridis*. *Animal Behaviour* 38:360-362.
- Graves, B. M. 1989. Defensive behavior of female prairie rattlesnakes (*Crotalus viridis*) changes after parturition. *Copeia* 1989:791-794.
- Greene, H. W. 1973. Defensive tail display by snakes and amphisbaenians. *Journal of Herpetology* 7:143-161.
- , 1986. Natural history and evolutionary biology In: M. E. Feder and G. V. Lauder (eds.), *Predator-Prey Relationships: Perspectives and Approaches from the Study of Lower Vertebrates*, pp. 180-189. University of Chicago Press, Chicago.
- , 1988. Antipredator mechanisms in reptiles. In: C. Gans and R. B. Huey (eds.), *Biology of the Reptilia*, Vol. 16, pp. 1-152. Alan R. Liss, New York.
- , 1992. The ecological and behavioral context for pit viper evolution. In: J. A. Campbell and E. D. J. Brodie (eds.), *Biology of the Pit Vipers*, pp. 107-117. Selva, Tyler, TX.
- Hardy, Sr., D. L., and H. W. Greene. 1999. Surgery on rattlesnakes in the field. *Sonoran Herpetologist* 12:26-28.

- _____, and _____. 2000. Inhalation anesthesia of rattlesnakes in the field for processing and transmitter implantation. *Sonoran Herpetologist* 13:110-114.
- Hedgecock, K. 1992. Defensive behavior of eastern massasauga rattlesnake In: B. Johnson and V. Menzies, (eds.), International Symposium and Workshop on the Conservation of the Eastern Massasauga Rattlesnake *Sistrurus catenatus catenatus*, pp. 99-103. Metro Toronto Zoo, West Hill, Ontario, Canada.
- Kissner, K. J., M. R. Forbes, and D. M. Secoy. 1997. Rattling behavior of prairie rattlesnakes (*Crotalus viridis viridis*) in relation to sex, reproductive status, body size, and body temperature. *Ethology* 103:1042-1050.
- Klauber, L. M. 1972. Rattlesnakes Their Habits, Life Histories, and Influence on Mankind. Vol. 1. University of California Press, Berkeley, CA.
- Knight, A., D. Styler, S. Pelikan, J. A. Campbell, L. D. Densmore, and D. P. Mindrell. 1993. Choosing among hypotheses of rattlesnake phylogeny: a best-fit rate test for DNA sequence data. *Systematic Biology* 42:356-367.
- Lehmann, V. W. 1969. Forgotten legions: sheep in the Rio Grande Plain of Texas. Texas Western Press, El Paso, 226 pp.
- Marshall, S. D., E. M. Jakob, and G. W. Uetz. 1999. Re-estimating fitness: can scaling issues confound condition indices? *Oikos* 87:401-402.
- Moon, B. R. 2001. Muscle physiology and the evolution of the rattling system in rattlesnakes. *Journal of Herpetology* 35:497-500.
- Place, A. J., and C. I. Abramson, 2004. A quantitative analysis of the ancestral area of rattlesnakes. *Journal of Herpetology* 38:152-156.

- Prior, K. A., and P. J. Weatherhead. 1994. Responses of free-ranging eastern massasauga rattlesnakes to human disturbance. *Journal of Herpetology* 28:255-257.
- Rowe, M. P., T. M. Farrell, and P. G. May. 2002. Rattle loss in pygmy rattlesnakes(*Sistrurus miliarius*): causes, consequences, and implications for rattle function and evolution, In: G. W. Schuett, M. Hoggren, M .E. Douglas, and H. W. Greene, (eds.), *Biology of the Vipers*, pp. 385-404. Eagle Mountain Publishing, Eagle Mountain, UT.
- Schaeffer, P. J. K. E. Conley, and S. L. Lindstedt. 1996. Structural correlates of speed and endurance in skeletal muscle: the rattlesnake tail shaker muscle. *Journal of experimental Biology* 199:351-358.
- Schultz, E., A. W. Clark, A. Suzuki, and R. G. Cassens. 1980. Rattlesnake shaker muscle: I. A light microscopic and histochemical study. *Tissue and Cell* 12:323-334.
- Secor, S. M., and T. R. Nagy. 2003. Non-invasive measure of body composition of snakes using dual-energy x-ray absorptiometry. *Comparative Biochemistry and Physiology Part A* 136:379-389.
- Seigel, S., and N. J. Castellan, Jr. 1988. *Nonparametric Statistics for the Behavioral Sciences*. 2nd Ed. McGraw-Hill, Boston, MA.
- Sweet, S. S. 1985. Geographic variation, convergent crypsis and mimicry in gopher snakes (*Pituophis melanoleucus*) and western rattlesnakes (*Crotalus viridis*). *Journal of Herpetology* 19:55-67.

- Young, B. A., and A. Aguiar 2002. Response of western diamondback rattlesnakes (*Crotalus atrox*) to airborne sounds. Journal of Experimental Biology Vol. 205: 3087-3092.
- Zimmerman, A. A., and C. H. Pope. 1948. Development and growth of the rattle of rattlesnakes. Fieldiana Zoology 32:357-413.

CHAPTER III

THE RATTLEBOX: AN APPARATUS FOR STUDYING LEARNING IN RATTLESNAKES

This article describes the rattlebox, an apparatus designed to study learning in rattlesnakes (genera *Crotalus* and *Sistrurus*). The rattlebox is relatively inexpensive, simple to construct, and can be adapted to study several learning paradigms. First, I describe the construction of an automated box that consistently induces rattling. Second, I report preliminary results of a study of habituation of the rattling response in three western diamondback rattlesnakes (*Crotalus atrox*). Finally, I suggest potential uses of the apparatus for studies of classical and operant conditioning.

Learning can be defined as a “relatively permanent change in behavioral potential as a result of experience (Abramson, 1994).” Countless studies have been published which assess the learning capacities of gold fish (*Carassius auratus*), pigeons (*Columbia livia*), white rats (*Rattus norvegicus*), and dogs (*Canis familiaris*) under various learning paradigms ranging from habituation to classical conditioning and operant procedures. While much has been discovered about learning processes using these traditional “laboratory species,” a true phylogeny of learning will require experiments with a greater number of non-traditional species (Bitterman, 1965). Further, behavior (and performance in learning tasks) can be used as characters in phylogenetic analysis (de Quieroz and Wimberger, 1993), which by its very nature requires a much broader taxonomic scope.

Snakes are one such group in which noticeably few learning studies have been published. There are probably several reasons for this paucity.

Burghardt (1977) noted the difficulties encountered in performing learning experiments with snakes. Among these difficulties are their relative inactivity (snakes spend a large proportion of their time sedentary), a general lack of positive reinforcement (most snakes consume large meals infrequently, [although see Burghardt et al. 1973, Holtzman et al., 1999; Kleinginna, 1970; and Begun et al., 1988; for exceptions]), and a limited number of dependent variables in which to measure (the tubular body form of snakes suggests a rather simple behavioral repertoire relative to other vertebrates).

Rattlesnakes are noticeably lacking from studies of learning probably due to the limitations listed above along with the relatively large body size of some species and handling difficulties associated with their venomous nature. One dissertation (Mills, 1970) describes attempts to use escape and avoidance contingencies with sidewinder (*C. cerastes*) and Mojave (*C. scutulatus*) rattlesnakes. Mills concluded that while head movements that allowed the snake to escape the shock were subject to change, true avoidance was not trainable in his three subjects. It should be noted that in studies of avoidance and escape it is important to consider the study animal's "species-specific defensive response" (Bolles, 1970). Thus, dependent variables appropriate for rattlesnakes should include occurrence of rattling, latency to rattling, and duration of rattling, along with the proto-typical striking and flight behavior, which have been utilized in previous learning studies of colubrids. With the first three dependent variables in mind, I have designed an apparatus that consistently releases the rattling response and allows for easy quantification of latency and duration.

The purpose of the rattlebox is to repeatedly apply an invariable stimulus to a test subject that consistently results in the subject rattling its tail. Previous studies have used stuffed animals (LaDuc, 2002), predator effigies (Shipman, 2002), or a human observer (Duvall et al., 1985) to elicit defensive behavior from rattlesnakes. However, because the experimenter presents each of these, there is variation among presentations in the way they are perceived by the test subject. Additionally, whenever a human observer serves as the stimulus, he or she risk being envenomated by the test subject. The apparatus diagrammed in Figure 3.1 reduces inter-presentation variation and the risk to the experimenter of being bitten.

Apparatus construction

The rattlebox is a simple 60 cm (L) x 64 cm (W) x 60 cm (H) box in which a moveable top is affixed to heavy-duty cabinet drawer slides that allow it to slide open horizontally. To reduce the possibility of pheromones influencing behavior, I built the prototype out of plywood covered with latex paint. I also covered the bottom of the rattlebox with newsprint that was changed following each training session. If pheromones are an issue, or the object of investigation, I suggest that the plywood be replaced with Plexiglas. Plexiglas is easier to clean and less likely to be contaminated by potentially confounding odors.

A snake is introduced into the apparatus through a 6.5 cm diameter keyhole located at the lower center of one side of the box. Snakes are coaxed into the box with a snake hook via this opening, which is closed with a swinging door. A clear acrylic barrier is located on the inside ca. 18 cm from the floor of the box to prevent snakes from escaping or being caught in the door as it returns to the closed position. The barrier has a

removable portion to facilitate cleaning and handling of subjects. The inside walls (above the acrylic barrier) and the underside of the moving door are covered with open-cell foam (5 cm thickness), which absorbs some of the noise made by the motor (see below) and the movement of the drawer slides. Three compression springs (one of which is mounted to the stop contact; see below) are glued to the leading edge of the door to dampen the force upon closing.

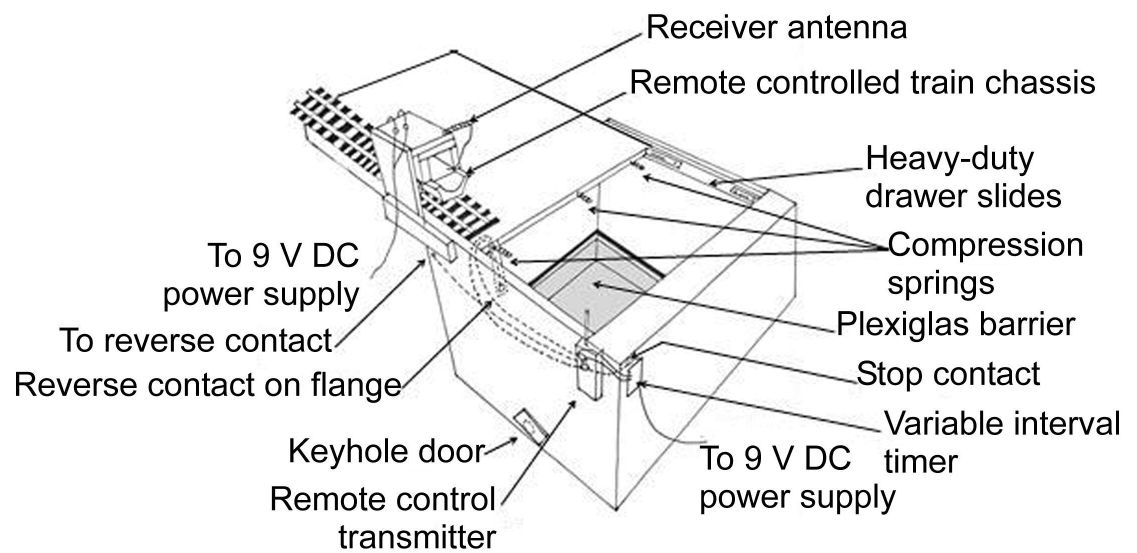


Figure 3.1. Schematic representation of the rattlebox. Note the compression spring on the stop contact positioned on the top of the flange bearing the reverse contact. (—) indicates wiring for the forward direction (i.e., open). (----) indicates wiring for the reverse direction (i.e., closed). (· · ·) indicates wiring to stop the motor.

Apparatus automation

The rattlebox is automated to open and close automatically at preset intervals using the motor of a remote-controlled toy train (Coastal Express, Scientific Toys, LTD, Chai Wan, Hong Kong), four electrical contacts and a cyclic timer (Canakit, Vancouver,

BC, Canada. <http://www.canakit.com>). Whereas I used a toy train motor to open and close the lid, any radio-controlled motor with forward and reverse will work.

The motor is fixed in a horizontal position with the wheels in contact with a track that is attached to the moving top (Figure 3.1). When the motor is engaged in the forward direction the box top is drawn open. The motor is engaged in the forward direction by two wires that connect the forward button of the remote controller to the normally open and common contacts on the interval timer. To return the top to the closed position, the motor is engaged in the reverse direction by contact made by copper contacts attached to a downward projecting flange affixed to the moving door and the stationary back wall. Contacts are soldered to wires that lead to the reverse button on the circuit board of the remote controller. The motor is stopped at the end of a trial by two additional contacts attached to the forward moving edge of the door and the inside of the front wall of the box. These contacts are soldered to wires that lead to the stop button on the circuit board of the remote controller. A parts list is available from the author upon request.

A microphone (Earthworks model # M30, Milford, NH) placed inside the center of the box and pre-amplifier (Earthworks model #1021, Milford, NH) outside the box are controlled via a laptop computer by an observer in an adjacent room. Just prior to the beginning of a trial, the observer starts a digital recorder (I used Audio Record Wizard <http://www.nowsmart.com/arwizard>) and gets a digital recording of each trial. Between trials each recording is analyzed and the occurrence/non-occurrence of rattling (along with other relevant variables) is recorded.

The rattlebox can be used in the implementation of multiple learning procedures. Below I give a preliminary description of its utility in the study of habituation of the rattle

response in western diamondback rattlesnakes. I also outline its potential uses in classical and operant conditioning studies.

Three wild-caught *C. atrox* from Oklahoma were used to test the rattlebox in a study of habituation of the rattle response. To produce habituation of the rattling response, the top of the box was opened every five minutes (i.e., intertrial interval = 5 min) automatically with the interval timer. For each trial, occurrence or non-occurrence of rattling was determined from playback of digital recordings. Habituation was achieved when a snake failed to rattle for ten consecutive trials, which is a strict criterion for behavioral studies (Ashmead and Davis 1996). Midway between the tenth consecutive no response trial and the eleventh trial, snakes were prodded with a snake hook through the keyhole for approximately 5 sec. This prod served as a dishabituating stimulus. Dishabituation is used to rule out effector fatigue and sensory adaptation as explanations for the observed response decrement to repeated stimulation (Abramson, 1994). Two and a half minutes following the presentation of the prod, the lid was opened and the presence or absence of rattling was recorded. The presence of a rattling response during the re-introduction of lid opening is evidence for a learned change in behavior (i.e., habituation).

Results of testing-The habituation curves of the rattle response for subjects D1, D3, and D12 are shown in Figure 3.2. Note the high probability of response in the first 50 trials for all three individuals ($\bar{X} \pm SE = 0.99 \pm 0.007$ for all three individuals combined). Following the dishabituating stimulus, all snakes responded. It is evident that the rattlebox is useful in consistently eliciting a rattle response and in detecting individual differences in rattling behavior.

Discussion

In addition to habituation, the rattlebox can also be used to study classical conditioning in rattlesnakes. I have just begun to test classical conditioning in rattlesnakes in which the opening and closing of the box serves as an aversive unconditioned stimulus. To serve as a conditioned stimulus, I have mounted a 60 W incandescent light on the inside of the box. Temporarily turning on the light serves as a conditioned stimulus.

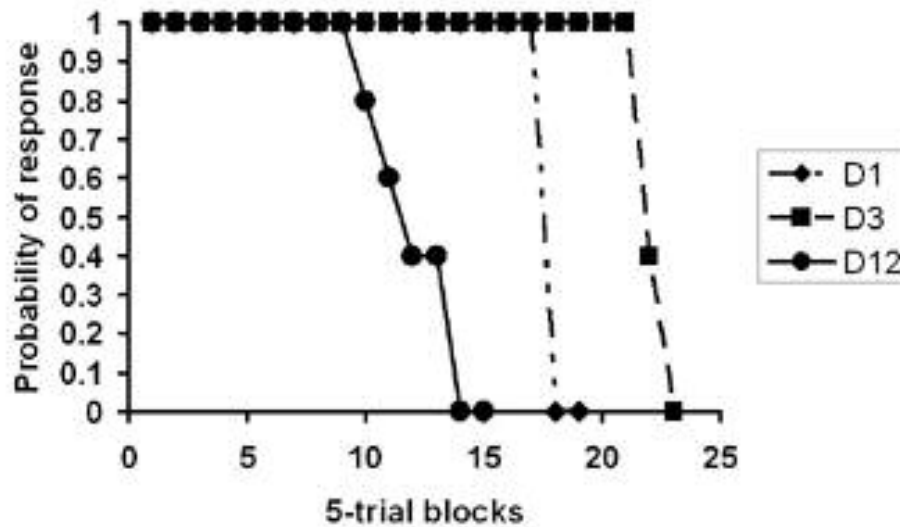


Figure 3.2. Habituation of the rattle response by three *Crotalus atrox* tested in the rattlebox. Note the ordinate is in 5-trial blocks and that some symbols overlap.

I have also designed an operant procedure that utilizes the rattlebox. In operant conditioning, a subject's response is reinforced by its consequences. By placing the rattlebox in a cold room and positioning a heat lamp inside wired to a voice-activated

relay, each rattle response is rewarded with an increase in ambient temperature. Since rattling typically does not occur spontaneously, it can be shaped by gently prodding the snake with a snake hook through the keyhole.

The results presented here demonstrate the utility of the rattlebox in studies of learning in rattlesnakes. Specifically, western diamondback rattlesnakes repeatedly exposed to an aversive yet harmless stimulus show a learned decrease in rattling. Furthermore, the box is relatively inexpensive and can be built in one day. Extension of the rattlebox to studies of classical and operant conditioning is also easily accomplished. I hope this apparatus will shed light on the perceptual world of rattlesnakes and stimulate further research in the area of learning in snakes.

Literature cited

Abramson, C. I. 1994. *A Primer of Invertebrate Learning: The Behavioral Perspective*.

American Psychological Association, Washington, D.C.

Ashmead, D. H., and D. L. Davis. 1996. Measuring habituation in infants: an approach using regression analysis. *Child Dev.* 67:2677-2690.

Begun, D., J. L. Kubie, M. P. O'Keefe, and M. Halpern. 1988. Conditioned discrimination of airborne odorants by garter snakes. *J. Comp. Psychol.* 102:35-43.

Bitterman, M. E. 1965. Phyletic differences in learning. *Am. Psychol.* 20:396-410.

Bolles, R. C. 1970. Species-specific defensive reactions and avoidance learning. *Psychol. Rev.* 77:32-48.

Burghardt, G. M. 1977. Learning processes in reptiles. In C. Gans & D. Tinkle (eds.), *The Biology of the Reptilia*, Vol. 7, pp. 555-681. Academic Press, New York.

- Burghardt, G. M., H. C. Wilcoxon, and J. A. Czaplicki. 1973. Conditioning in garter snakes: aversion to palatable prey induced by delayed illness. *Anim. Learn. Behav.* 1:317-320.
- de Quieroz, K., and P. H. Wimberger 1993. The usefulness of behavior for phylogeny estimation: levels of homoplasy in behavioral and morphological characters. *Evolution* 47:46-60.
- Duvall, D., M. B. King, and K. J. Gutzwiller. 1985. Behavioral ecology and ethology of the prairie rattlesnake. *Nat. Geog. Res.* 1:80-111.
- Holtzman, D. A., T. W. Harris, G. Aranguren, and E. Bostock. 1999. Spatial learning of an escape task by young corn snakes, *Elaphe guttata guttata*. *Anim. Behav.* 57:51-60.
- Kleinginna, P. R., Jr. 1970. Operant conditioning in the indigo snake. *Psychon. Sci.* 18:53-55.
- LaDuc, T. J. 2002. Does a quick offense equal a quick defense? Kinematic comparisons of predatory and defensive strikes in the western diamondback rattlesnake (*Crotalus atrox*). In G. W. Schuett, M. Hoggren, M. E. Douglas, and H. W. Greene (eds.), *Biology of the Vipers*, pp. 267-278. Eagle Mountain Publishing, Eagle Mountain, UT.
- Mills, K. C. 1970. Attempts at escape and avoidance training with the rattlesnake using infrared light as the discriminative stimulus. Ph.D. dissertation. Claremont University, Claremont, California. 57 pp.

Shipman, P. A. 2002. The evolution of antipredator behavior in snakes: ultimate and proximate determinates. Unpubl. Ph.D. diss., Oklahoma State University, Stillwater. 166 pp.

CHAPTER IV

HABITUATION OF THE RATTLE RESPONSE IN WESTERN DIAMONDBACK RATTLESNAKES

Habituation can be defined as a decrement of response to an iterative stimulus. Habituation is one of the simplest types of learning (Thorpe, 1963) and is adaptive in several regards (Abramson, 1994). First, habituation restricts defensive behavior to infrequently occurring stimulus patterns. Second, it conserves energy by reducing the occurrence of exploratory and defensive behaviors. Third, habituation allows species-typical behavior to occur in otherwise unpleasant situations

Habituation probably occurs throughout the animal kingdom (Mazur, 1986). Despite this ubiquity, few studies specifically addressing habituation have been conducted using snakes. This paucity of studies using snakes may stem from a lack of interaction among comparative psychologists and herpetologists, or the perceived lack of a complex behavioral repertoire in snakes.

Fuenzalinda et al. (1975) showed a decrease in defensive body movements by the garter snake, *Thamnophis radix*, to the shadow of a human hand over fifteen trials. Herzog et al. (1989) are the only workers to investigate the effect of both long-term and short-term habituation. They found significant habituation of striking by *T. melanogaster* over five days, but not by *T. butleri* over the same time period. *T. melanogaster* also exhibited a significant decline in strikes over ten consecutive stimulus presentations 30

sec apart. Hampton and Gillingham (1989) showed that neonate garter snakes (*T. sirtalis*) habituate more readily to overhead stimuli than to stimuli presented at eye level. The only pit viper to be examined for habituation is the cottonmouth moccasin (*Agkistrodon piscivorus*). Defense scores declined significantly over five daily trials (Glaudas, 2004). The only study to address habituation of a feeding response found a decline over 15 trials of tongue-flick attack scores in water snakes (*Nerodia rhombifer*) responding to the presentation of minnow odors (Czaplicki, 1975). None of these studies addressed individual variation in habituation. Fuenzalinda, et al. (1975) merely commented on the large amount of inter-individual variation. Glaudas (2004) presented individual overall mean responses, but did not show the response trajectory of each individual over the five successive test days. This lack of individual data is perplexing since habituation is an individual phenomenon.

Rattlesnakes are noticeably lacking from studies of habituation despite the presence of several useful behavioral responses for such studies. The rattlesnake rattle presumably indicates the snake's presence to potential predators (Greene, 1988). The rattle sound is an audible hiss or buzz in the frequency range 2-20 khz (Fenton and Licht, 1990) and transmits information coded in the duration, frequency, and intensity of the sound. Easily measured dependent variables include presence/absence of rattling, duration and intensity of rattling, and latency to rattle,.

In this study, I begin parameterization of habituation of the rattling response. First, I demonstrate that the rattle response exhibits both short-term and long-term habituation. I also determine the utility of several dependent variables in measuring habituation and I make recommendations for future studies of habituation.

Methods

Study animals

Ten wild-caught western diamondback rattlesnakes (4 males, 6 females) from Blaine and Major, counties, OK, were used in this experiment. Snakes were collected from April to November, 2003. Mean mass and snout-to-vent length (± 1 SE) of the snakes were, respectively, 80.20 ± 4.96 cm and 375.08 ± 74.2 g. Snakes were held in captivity 6-8 months prior to testing. A sample size of 10 was used because I was interested in individual performance. Measuring individual performance for more than 10 individuals was impractical in the present study because of the large number of trials required to document habituation (see below). Snakes were maintained individually in large plastic storage containers at $25.6 \pm 3^\circ$ C. Light:dark cycle was approximately 8h:16h (except during a winter cool period, 0h:24h). Food was offered weekly (except during the winter cool period) and water was available *ad libitum*. During December and January, two months prior to testing, snakes were maintained at $15 \pm 2^\circ$ C for four weeks to simulate the winter cool down period during. An animal care and use protocol is on file with the Oklahoma State University Animal Care and Use Committee (Protocol #AS0317).

Procedure

To elicit rattling behavior I used the rattlebox as described in Chapter Three. Essentially, it is a box with a sliding top that opens and closes automatically at regular intervals. When the box opens, the snake located inside the box rattles its tail. For my study, the rattlebox was programmed to open automatically with an intertrial interval of five minutes. Each trial was recorded with a digital recording system placed inside the

box. Snakes were allowed to acclimate to the box for 5-10 minutes prior to the first box open-close sequence.

Snakes were subjected to the box opening and closing until they reached the criterion of no response in 10 consecutive trials or until 120 trials were completed. A criterion of ten no-response results is very stringent. Hampton and Gillingham (1989) used a criterion of four consecutive no response trials on each of their testing days. Most studies of habituation use a proportional decrease (usually 70%) in response magnitude relative to the previous maximum response magnitude (Ashmeed and Davis, 1996). To rule out a response decrement due to effector fatigue or sensory adaptation, two and one-half minutes following the tenth no response, a subject was prodded with a snake hook (>5 sec) to elicit rattling. Two and one-half minutes after this dishabituating prod, the original box opening stimulus was presented again, and the rattle response recorded. Following testing, the snake was returned to its home container and replaced in the snake room until the following day when it was tested in a manner identical to that of the previous day. All snakes were tested on four consecutive days. The rattlebox was cleaned and newsprint substrate was replaced daily. Only one snake was tested per day.

For each opening of the box, I recorded presence/absence of rattling, latency to rattle (time from initial box opening to onset of rattling), duration of rattling (time from onset of rattling to cessation of rattling), and end duration (time from box closed to rattle cessation).

Statistical analysis

Habituation within and between individuals - To quantify habituation within and between individuals, I used as a qualitative measure the number of trials to reach criterion

each day. The occurrence of dishabituation was also determined for each individual on each day, following performance to criterion. I also compared the slopes of individual regressions (Petrinovich and Widman, 1984) for each snake on each day by using 95% confidence limits for latency, duration and end duration. The slope of the individual regression will be negative and indicates the rate at which habituation occurs. On successive training days one expects to see an increase in the slope (habituation occurring faster). The occurrence/non-occurrence data were analyzed using logistic regression. The odds ratio interpretation of the beta coefficient of the logistic regression equation is cumbersome, so instead I calculated the trial at which there was a 50% probability of a rattle response to occur. This corresponds to the position on the x-axis where the regression line is the steepest (Agresti, 1996). I assumed if the rate of habituation was increasing (within individuals over days) or was faster (between individuals within a day) this steepest slope value should occur after fewer trials.

Habituation within the group - For the test group, I performed repeated measures ANOVA on the first 20 trials of each day for latency, duration, and end duration. To compare habituation between days, I performed repeated-measure ANOVA on the number of trials to criterion and the savings scores over four days. The savings score was calculated by subtracting the number of trials to criterion on day two from the number to criterion on day one, and subtracting day three from day two, and so on. I also performed repeated measures ANOVA on the individual's daily means for latency, duration, end duration and the steepest slope trial number over the four test days. Any significant ANOVA was further analyzed with polynomial contrasts to detect significant trends. The Huynh-Feldt adjustment to the degrees of freedom was applied to all repeated measures

analyses (Kuehl, 2000). Cochran's Q test was used to test the dichotomous repeated measures data (Siegel and Castellan, 1988).

Habituation of latency results in increased latency (i.e., snakes take longer to initiate rattling) over trials. Hence, the reciprocal of latency was used in all analyses so all of the dependent variables followed the same decreasing pattern. Nonetheless, I will refer to it as latency throughout the paper. Latency, duration and end duration were natural-log transformed before analysis.

Results

Habituation within and between individuals– Number of trials to criterion varied markedly within individuals between days and between individuals within days (Figure 4.1). Of the 40 test days (10 snakes tested on four days each), 10 days did not end with the subject reaching criterion. Four snakes are responsible for these 10 days. Snake D6 never went to criterion on any test day. Snake D10 went to criterion only on Day 4. Snake D2 went to criterion on Days 1 & 2, but not 3 & 4. Snake D4 went to criterion on Days 2, 3, & 4. Three of these snakes (D4, D6, & D10) each rattled 120 consecutive times at five-minute intervals.

Two individuals did not exhibit dishabituation on some days following the prod with a snake hook after the tenth no-response trial (Figure 4.1). Snake D1 did not respond on day four after reaching criterion. Snake D5 did not respond on days 1, 2, and 3 after reaching criterion. However, each snake did rattle each time it was prodded with the snake hook. The habituation curves for all four dependent variables are shown in figures 4.2-4.5.

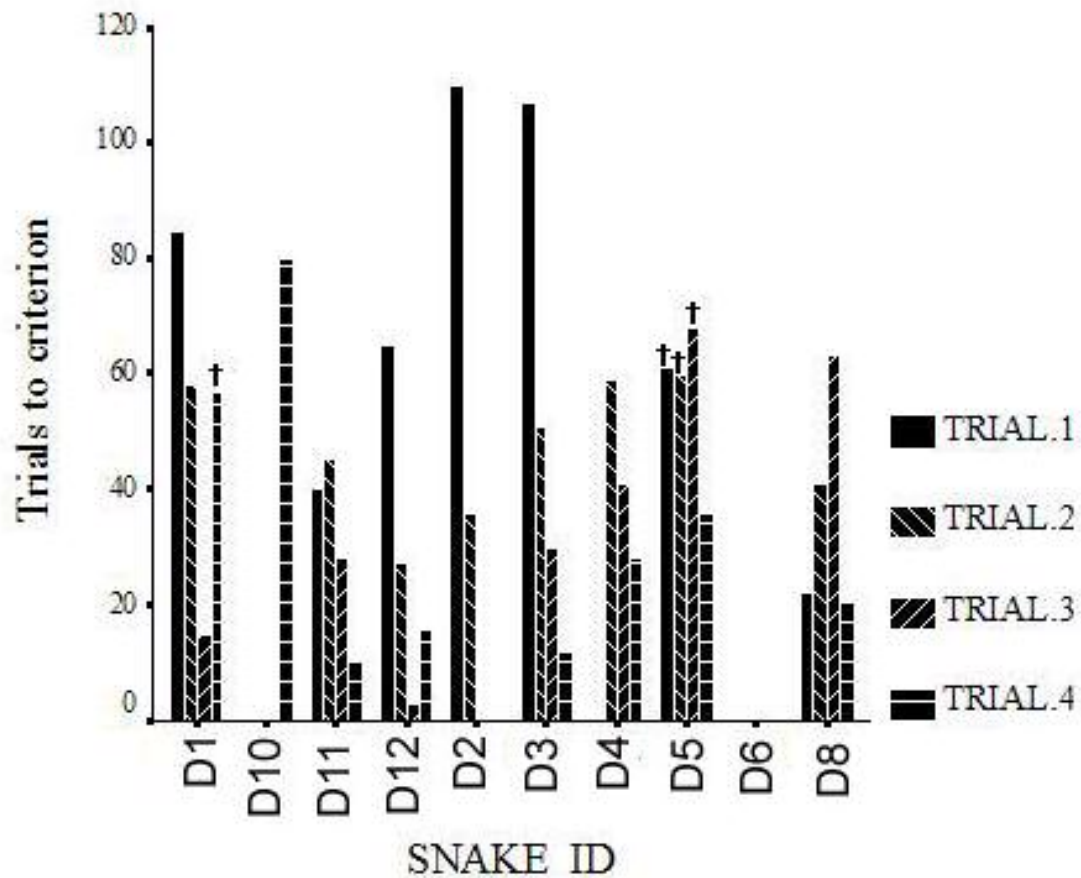


Figure 4.1. The number of trials required for ten snakes to reach the 10 consecutive no-rattle response criterion on four consecutive days. Intertrial interval for stimulus presentation was 5-minutes.

† indicates individual days in which a snake did not rattle following a dishabituation prod with a snake hook

The probability to rattle when the rattlebox opened tended to remain high (0.8-1.0) early in a testing day, followed by a rapid decline to zero (figure 4.2). However, there was considerable variation between individuals on any given day and variation within individuals between days. Generally, the probability to rattle declined to zero more rapidly on successive days of training, though not all individuals showed this pattern. A

few snakes (e.g., D2, D5, D8, & D10) exhibited a “zig-zag” pattern in which the probability to rattle decreased to moderate or low (0.6-0.2) levels, then increased in probability to high levels before finally ending at a probability of zero.

The trial at which the steepest slope occurred in the individual regression exhibited considerable variation within individuals, although the general trend was for the steepest slope to occur earlier during testing on successive days (figure 4.3). A large amount of inter-individual variation was also evident. Some individuals had steepest slopes on the first day of training in under 50 trials, whereas other individuals has steepest slopes on the first day at over 80 trials (Figure 4.3).

The latency to rattle (plotted as the reciprocal) tended to decrease over successive trials within a day and decline more rapidly over successive days within individuals (Figure 4.4). Again, the variation within and between individuals, and within and between days, is marked. A few individuals (e.g. D2, D3, D6) showed an initial increase followed by a decrease to some asymptote before rattling ceased entirely. The initial magnitude of latency to rattling also differed between individuals. Some individual's initial responses (especially on the first day of training) were very high (e.g. D6, D10) whereas that of others was considerably lower (e.g. D2, D5, D11).

Duration of rattling and end duration also decreased over successive trials (Figures 4.4 & 4.5) and followed a pattern similar to those illustrated by probability to rattle and latency to rattle. Inter- and intra-individual variation is again apparent.

Most of the slopes for the latency to rattle were significantly less than zero and varied between individuals. There was no apparent pattern in the change in slope coefficients between days within individuals (Figure 4.7). Individual slope coefficients

for rattling duration were significantly negative and differed between individuals within days (Figure 4.7). The change in slope between days within individuals revealed no pattern and most individuals did not increase the rate of habituation (as measured by duration) between days. A majority of the slope coefficients for end duration were significantly negative and different between individuals and days (Figure 4.7). Most of the differences between days were between day 1 and subsequent days. End duration did not habituate at an increasing rate over subsequent testing days.

Habituation within the group – Repeated measures ANOVA over the first twenty trials for each day for latency was not significant on any of the four days (Table 4.1). Repeated-measures ANOVA with duration as the dependent variable showed a significant time effect on days one and two only (Table 4.1). When end duration was used as the dependent variable in the repeated-measures analysis only day 1 and day 2 showed a significant effect of time (Table 4.1). For the dichotomous dependent variable, rattle response, only day four had a difference among trials (Day 1: $Q = 17.17$, $df = 19$, $P = 0.578$; Day 2: $Q = 25.85$, $df = 19$, $P = 0.135$; Day 3: $Q = 14.52$, $df = 19$, $P = 0.753$; Day 4: $Q = 50.68$, $df = 19$, $P < 0.001$).

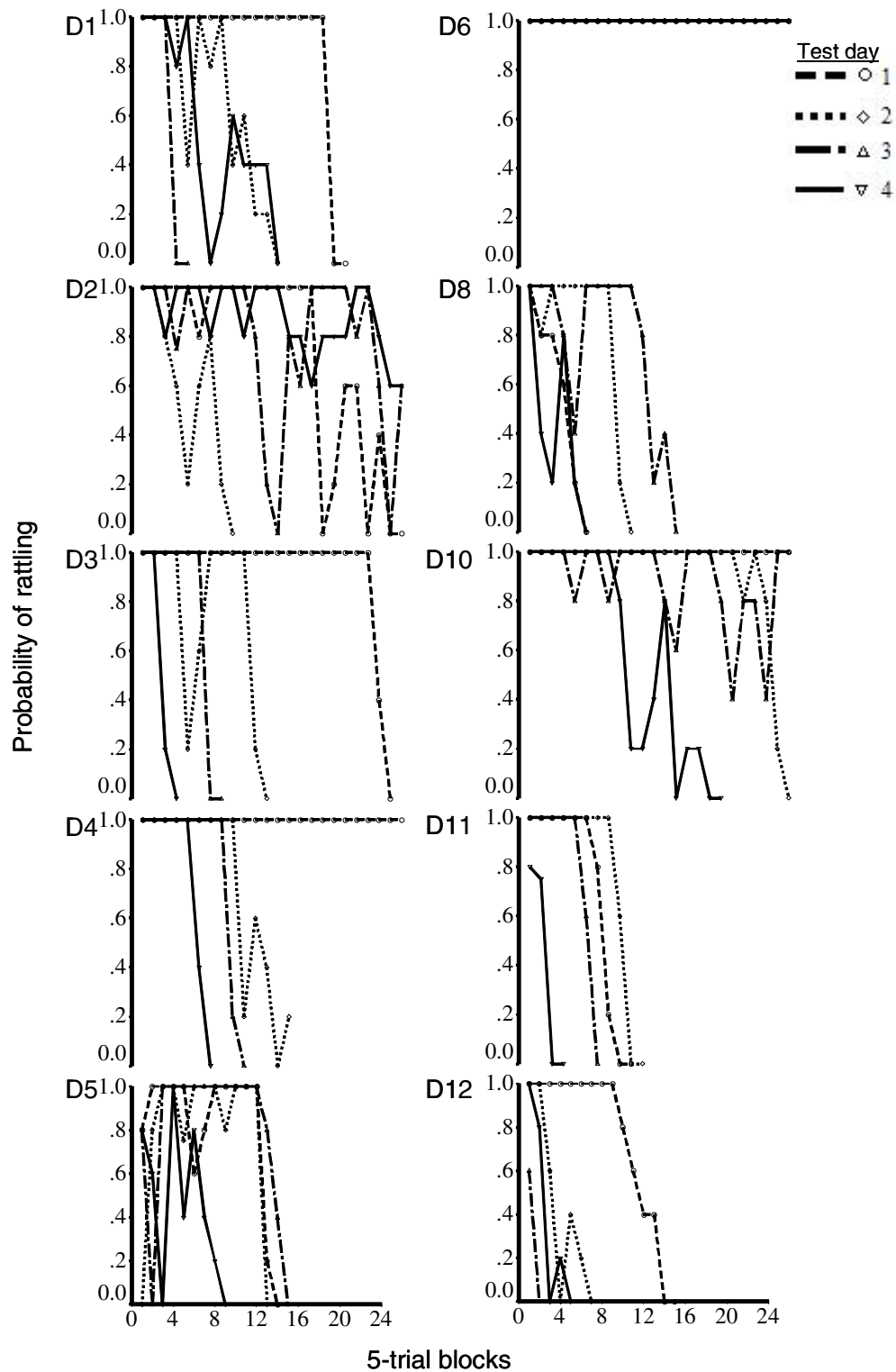


Figure 4.2. Habituation of the rattle response in ten individual western diamondback rattlesnakes. Note the x-axis is in five trial blocks and the y-axis is the probability to rattle in a given block.

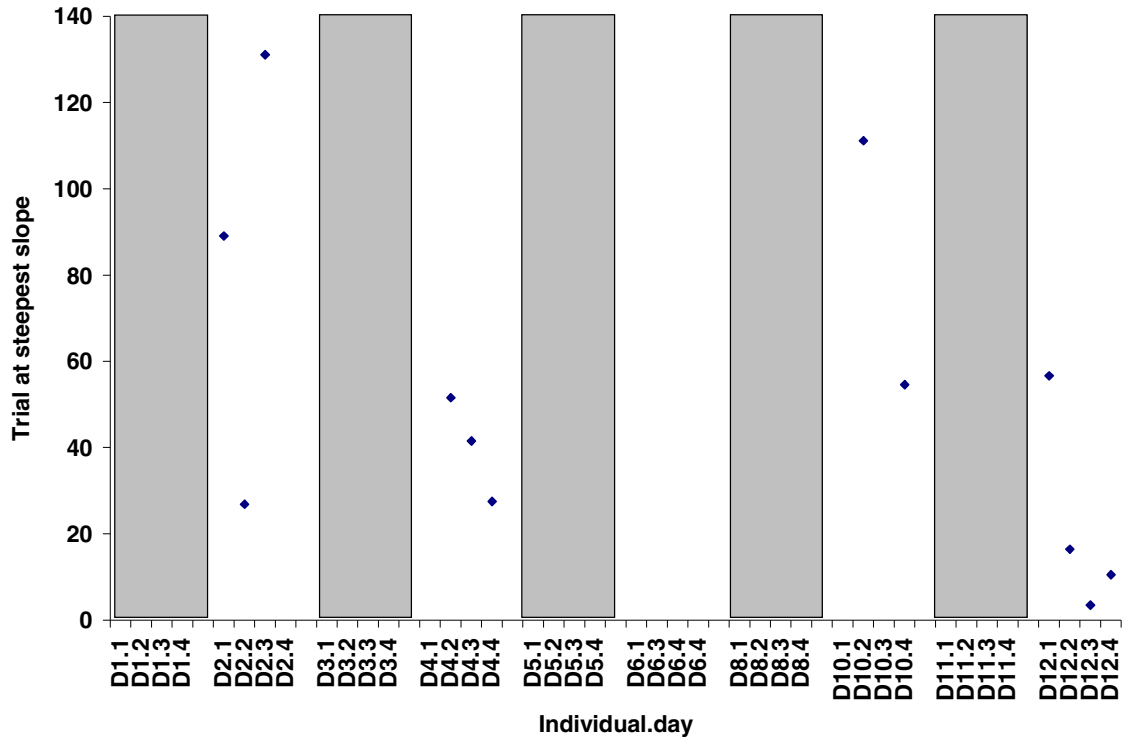


Figure 4.3. The trial at which the steepest slope of logistic regression occurred for 10 western diamondback rattlesnakes tested on four consecutive days. Non-significant regressions and snakes that did not habituate are not plotted on the figure.

Comparing responses within the group across days, repeated measures ANOVA on the number of trials to criterion revealed a significant effect of time (Table 4.2). The trial at which the steepest slope occurred in the logistic regression also exhibited a significant effect of days (Table 4.2) Analysis of individual daily means of latency across the four test days revealed a significant linear trend (Table 4.2). Similarly, duration also exhibited a significant time effect (Table 4.2). The analysis with end duration as the dependent variable was comparable to duration (Table 4.2).

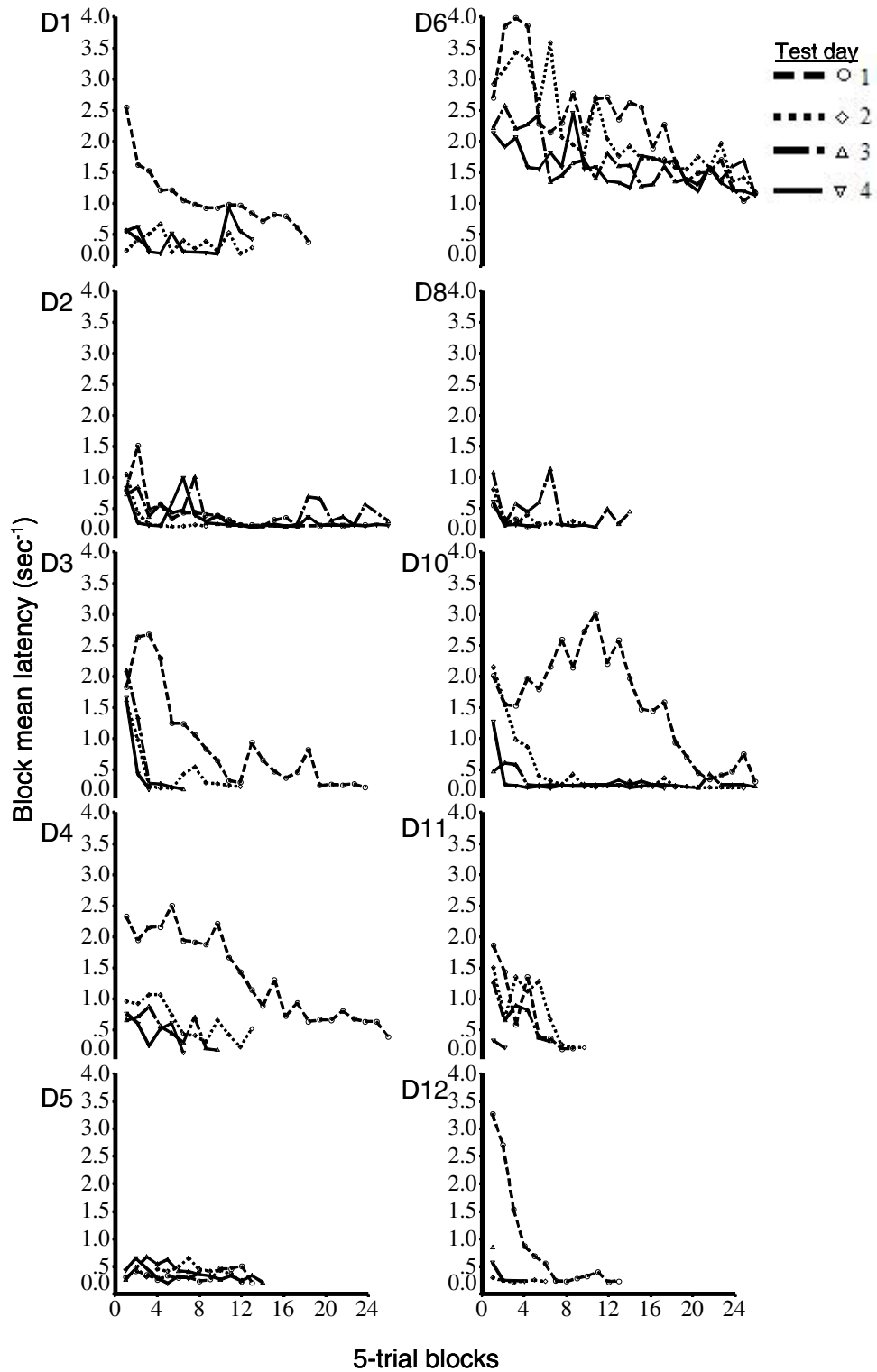


Figure 4.4. Habituation of latency to initiate rattling in ten western diamondback rattlesnakes. X-axis as in Figure 4.2

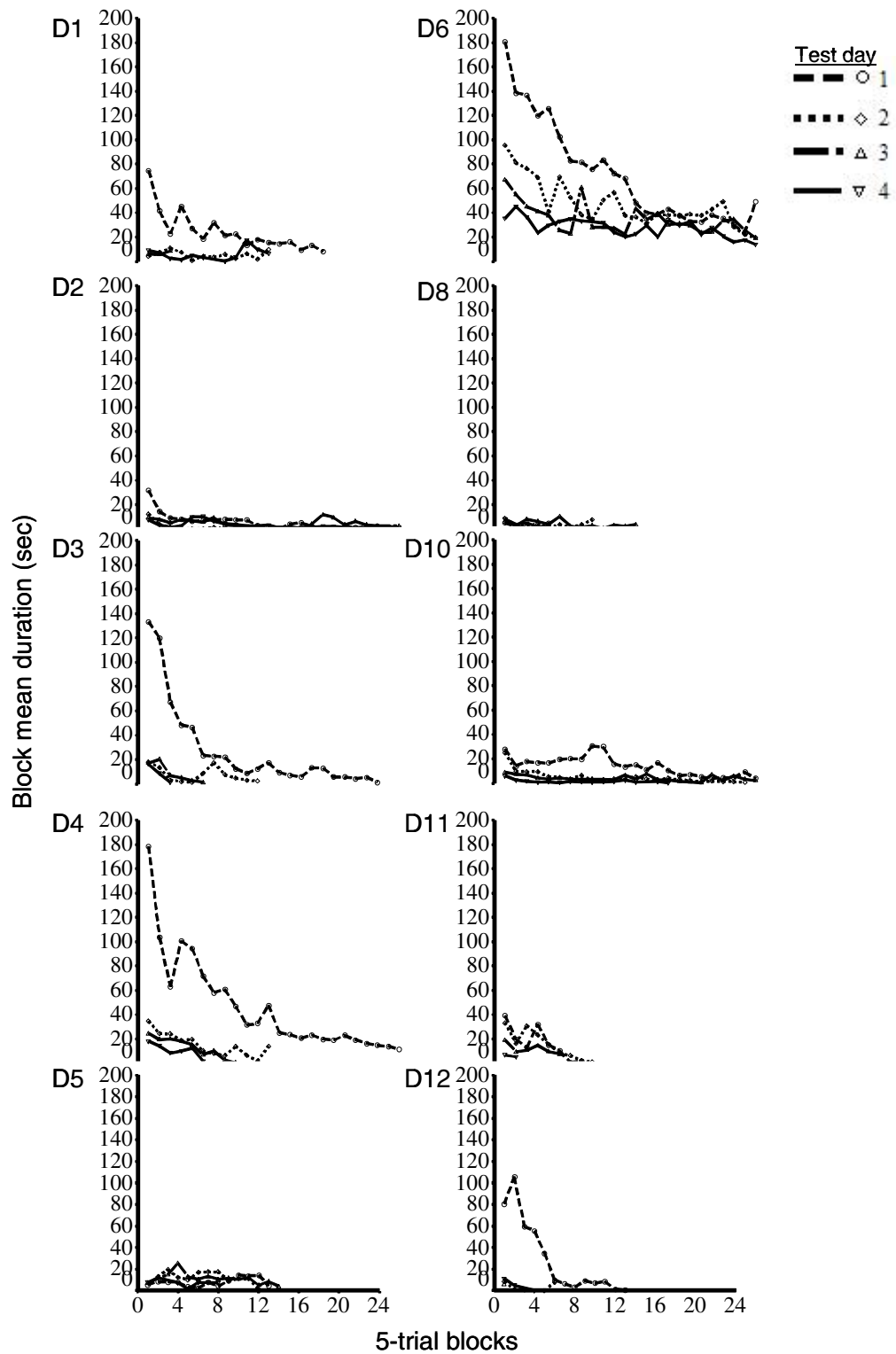


Figure 4.5. Habituation of the duration of rattling in ten western diamondback rattlesnakes. X-axis as in Figures 4.2 and 4.4.

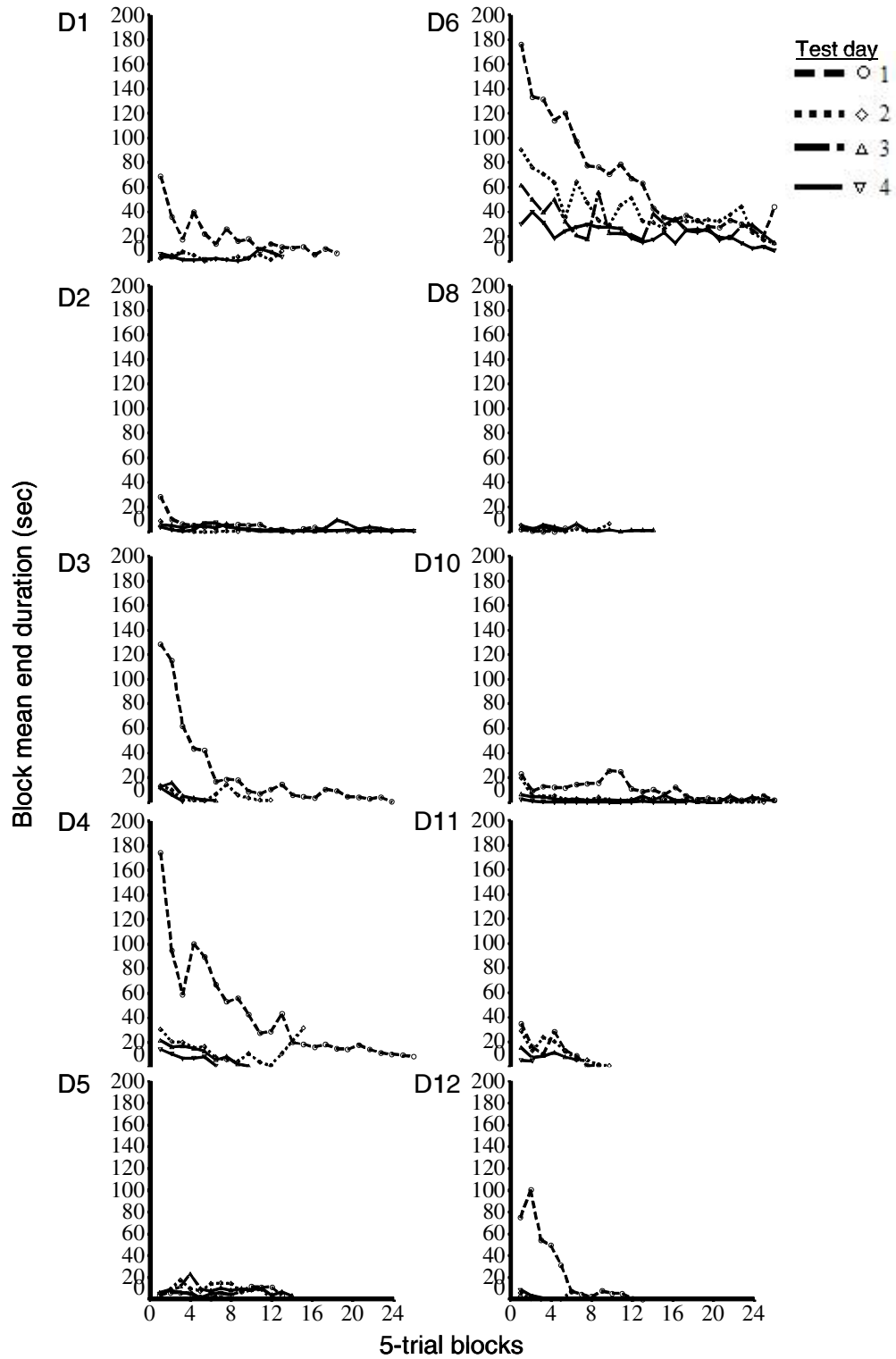


Figure 4.6. Habituation of the end duration of rattling (see text for definition) in ten western diamondback rattlesnakes. X-axis as in Figures 4.2.

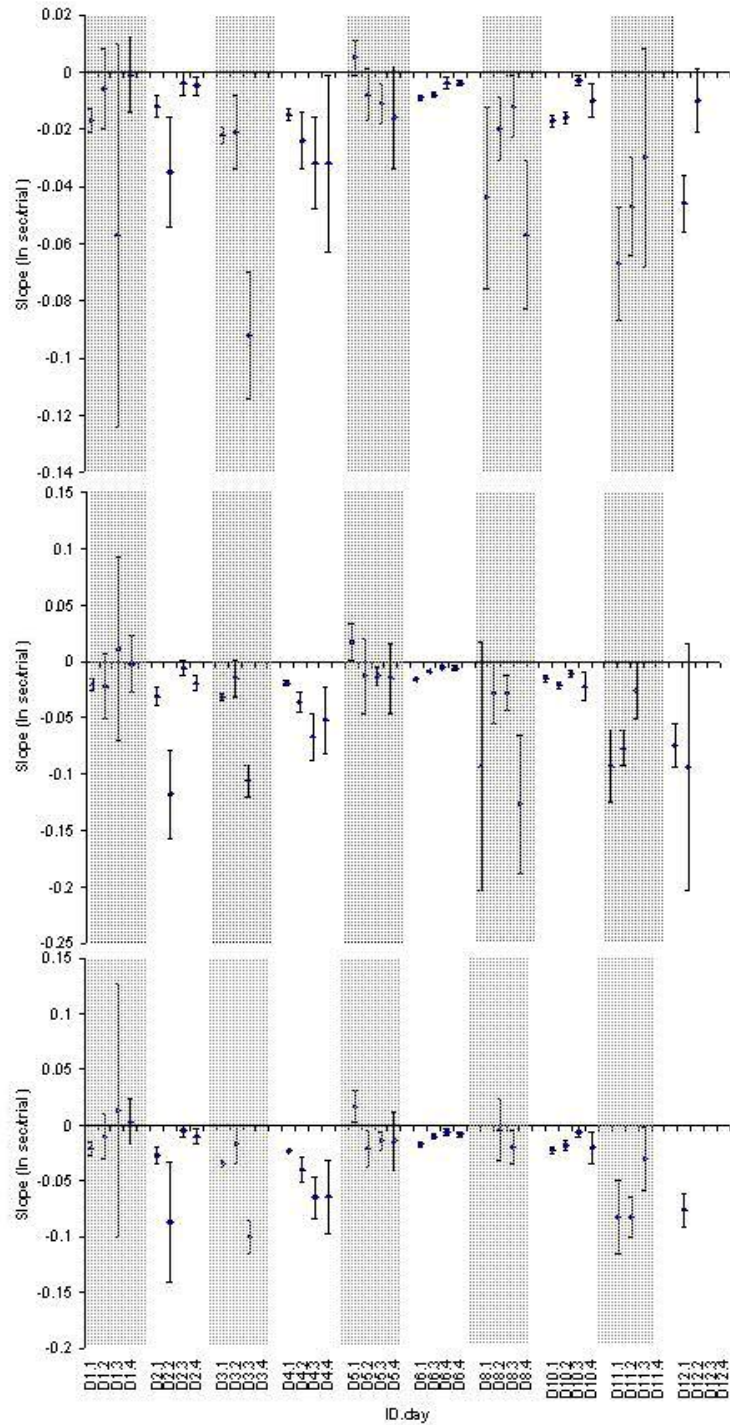


Figure 4.7. Regression slopes from individual regression analysis of three variables (from top to bottom: latency, duration, and end duration) associated with habituation of the rattle response in 10 western diamondback rattlesnakes. Regressions based on fewer than 10 data points were considered unreliable and are not included in the figure.

Table 4.1. Repeated-measures ANOVA table for three dependent variables measured over the first twenty trials on four consecutive days. Blank rows were not computed because of insufficient degrees of freedom.

Variable	Day	Contrast	DF	F	P	η^2	Post-hoc power
Latency	1		7.73, 38.64	0.714	0.673	0.125	0.277
	2						
	3		6.61, 13.22	1.25	0.344	0.384	0.341
	4						
Duration	1		6.05, 30.26	2.89	0.024	0.366	0.819
		Linear	1, 5	8.48	0.033	0.629	0.647
		Quadratic	1, 5	6.24	0.055	0.555	0.522
	2		3.94, 11.81	3.70	<0.0001	0.552	0.713
		Linear	1, 5	50.29	0.006	0.944	0.993
	3		5.98, 11.96	1.62	0.224	0.448	0.409
	4		1.40, 2.80	1.43	0.350	0.417	0.137
End duration	1		5.89, 29.46	2.86	0.026	0.364	0.806
		Linear	1, 5	8.13	0.036	0.619	0.629
		Quadratic	1, 5	6.44	0.052	0.563	0.534
	2		7.44, 27.74	3.36	0.008	0.456	0.918
		Linear	1, 4	14.91	0.018	0.788	0.819
	3		2.21, 8.83	1.28	0.330	0.242	0.215
	4		1.36, 2.72	1.25	0.381	0.385	0.124

Table 4.2. Repeated measures ANOVA table on six dependent variables measured over four consecutive days exhibiting long-term habituation.

Variable	Contrast	DF	F	P	η^2	Post-hoc power
Trials to criterion		3,15	3.32	0.049	0.399	0.635
	Linear	1, 5	8.10	0.036	0.618	0.628
Savings scores		2,12	0.346	0.714	0.055	0.093
Trial at steepest slope		3, 9	8.918	0.005	0.748	0.947
	Linear	1, 3	16.858	0.026	0.849	0.774
Mean latency		2.61, 23.49	6.48	0.002	0.419	0.918
	Linear			0.014	0.503	0.767
Mean duration		1.46, 13.14	10.55	0.003	0.540	0.922
	Linear	1, 9	12.09	0.007	0.575	0.871
	Quadratic	1, 9	6.68	0.029	0.426	0.635
Mean end duration		1.39, 12.54	9.77	0.005	0.521	0.890
	Linear	1, 9	10.82	0.009	0.546	0.883
	Quadratic	1, 9	6.61	0.030	0.423	0.630

Discussion

Habituation of the rattle response and several dependent variables associated with rattling were clearly demonstrated. This is not surprising since habituation of defensive responses have been noted in several other snake taxa including *Thamnophis radix*, *T. sirtalis*, *T. butleri*, *T. melanogaster*, and *A. piscivorus* (Fuenzalinda, et al., 1975; Hampton and Gillingham, 1989; Herzog et al., 1989; Glaudas, 2004). Most notable in the habituation of the rattle response is the high variability within and between

individuals. Some individuals exhibited rapid and linear declines in response probability whereas others exhibited a “zig-zag” pattern, and four snakes did not reach criterion on one or more test days (Figure 4.2). This variation might reflect unknown differences in prior experience of the test animals or differences in genetic predisposition toward habituation. Partitioning of the effects of genes and environment on habituation in rattlesnakes should be studied.

Two individuals did not rattle to the box opening following the dishabituating prod with the snake hook on one or more days. This probably was not a result of muscular fatigue or sensory adaptation because a rattle response occurred during the prod. This failure to respond was most likely the result of habituation “proceeding beyond zero” (Thompson and Spencer 1966). Recovery of the response to the original stimulus will be slow when an organism continues to receive a stimulus after its response has disappeared completely. Hence, in the snakes observed in this experiment, there may not have been enough recovery time following the tenth no-response for the rattle response to resume.

A hallmark of repeated habituation is a decreased initial response on successive days, also known as habituation of dishabituation (Thompson and Spencer 1966). Repeated-measures ANOVA showed a significant decreasing trend over test days for latency, duration and end duration. However, this pattern was not clear in several individuals. In *Aplysia californica*, habituation of dishabituation was shown when the gill withdrawal reflex was stimulated for just 10 trials on each of four consecutive days (Grew, et al ., 1972). By training animals to criterion, most animals received many more trials than ten. This may have allowed some individuals to key in on specific aspects of

the procedure (e.g., the door took ca. 3 seconds to open and 3 seconds to close), thereby allowing for one or more types of associative learning to become involved, such as classical conditioning, for example.

Another key aspect of repeated habituation is an increase in rate of habituation over successive days (Thompson and Spencer, 1966). While this was noted to some degree in the number of trials to criterion in four individuals, individual regression analysis on all four dependent variables did not reveal a pattern of increasingly negative regression slopes (Figure 4.7). Running the snakes to criterion may have had an impact on this phenomenon similar to the effect on habituation of dishabituation. However, analyzing habituation of the entire test group did show significant trends between days for number of trials to criterion, trial of steepest slope, latency, duration, and end duration. This discrepancy between individual and group data is common in studies of learning (Abramson, 1994). The difference between group and individual performance is the result of an overall decrease in variation in the dependent variable. That is, extreme values have less effect on the group data than on the individual data. Additionally, in the grouped data, unusually high values for one individual are often “cancelled out” by unusually low values for another.

I measured habituation of four behaviorally relevant components of the rattle response; occurrence, latency, duration, and end duration. These four measures differed in utility as measures of habituation. The probability of occurrence is not useful in measuring habituation over a limited number of trials (I arbitrarily assessed the effect of 20 trials in the present study) because it occurs too frequently for a decrement of response to be apparent. I would not recommend using the occurrence of rattling as the sole

dependent measure in studies of habituation in rattlesnakes because it is more difficult to handle statistically and does not show much change over a limited number of trials. Duration and end duration were redundant. I recommend utilizing duration as the dependent variable in future studies of habituation in rattlesnakes.

Running animals to criterion is useful in that it clearly elucidated individual variation and potential to rattle. My criterion of 10 no responses was extremely time consuming when coupled with my alternative criterion of a maximum of 120 trials before ending sessions. This combination occasionally resulted in training sessions that lasted 10 consecutive hours. I advocate one of two approaches. First, if a criterion is going to be used, relax it to fewer consecutive no response trials (e.g., 5-7). Alternatively, it may be appropriate to accept 7 of 10 trials resulting in no response as evidence of habituation as long as a dishabituating stimulus is used following the achievement of criterion. Alternatively, no criterion can be used and individual snakes can be measured over 20 trials on each of several consecutive days.

Future studies of habituation of the rattle response need to be conducted in other taxa before workers construct a phylogeny of habituation in rattlesnakes. Once this is accomplished it will be possible to evaluate hypotheses regarding the origin and evolution of the crotaline rattle. Moreover, in a broader context, once habituation in rattlesnakes has been fully parameterized, more complex forms of learning can be investigated, including classical and operant conditioning. This will allow us to add snakes to the phylogeny of learning begun by Bitterman (1975) over 40 years ago.

Literature cited

- Abramson, C. I. 1994. A Primer of Invertebrate Learning: The Behavioral Perspective. American Psychological Association, Washington, D.C.
- Ashmeed, D. H. and D. L. Davis. 1996. Measuring habituation in infants: an approach using regression analysis. *Child Development* 67:2677-2690.
- Bitterman, M. E. 1975. The comparative analysis of learning. *Science* 188:699-709.
- Carew, T. J., H. M. Pinsker, and E. R. Kandel. 1972. Long-term habituation of a defensive withdrawal reflex in *Aplysia*. *Science* 175:451-454.
- Czlapicki, J. 1975. Habituation of the chemically elicited prey-attack response in the diamondbacked water snake, *Natrix rhombifera*. *Herpetologica* 31:403-409.
- Fenton, M. B., and L. E. Licht. 1990. Why rattle snake? *Journal of Herpetology* 24:274-279.
- Fuenzalinda, C. E., G. Ulrich, and B. T. Ichikawa. 1975. Response decrement to repeated shadow stimuli in the garter snake, *Thamnophis radix*. *Bulletin of the Psychonomic Society* 5:221-222.
- Glaudas, X. 2004. Do Cottonmouths (*Agkistrodon piscivorus*) habituate to human confrontations? *Southeastern Naturalist* 3:129-138.
- Greene, H. W. 1988. Antipredator mechanisms in reptiles. Pp.1-152, In C. Gans and R. B. Huey (Eds.). *Biology of the Reptilia*. Volume 16. Alan r. Liss:New York, NY.
- Hampton, R. E., and J. C. Gillingham. 1989. Habituation of the alarm reaction of neonatal eastern garter snakes, *Thamophis sirtalis*. *Journal of Herpetology* 23:433-435.

- Herzog, H. A., B. B. Bowers, and G. M. Burghardt. 1989. Development of antipredator responses in snakes IV: interspecific and intraspecific differences in habituation of defensive behavior. *Developmental Psychobiology* 22:489-508.
- Kuehl, R. O. 2000. *Design of Experiments: Statistical Principles of Research Design and Analysis*. 2nd Ed. Duxbury Press:Pacific Grove, CA.
- Mauzer, J. E. 1986. *Learning and Behavior*. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
- Petrinovich, L., and K. F. Widman. 1984. An evaluation of statistical strategies to analyze repeated measures data. Pp. 155-201, In H. V. S. Peeke and L. Petrinovich (Eds.). *Habituation, Sensitization, and Behavior*. Academic Press:Orland, FL.
- Place, A. J., C.I. Abramson, and V. Ramirez. The rattlebox: an apparatus for studying learning in rattlesnakes. *Herpetological Review* (In Press)
- Seigel, S. and N. J. Castellan, Jr. 1988. *Nonparametric Statistics for the Behavioral Sciences*. 2nd Ed. McGraw-Hill:Boston, Mass.
- Thompson, R. F., and W. A. Spencer. 1966. A model phenomenon for the study of neuronal substrates of behavior. *Psychological Review* 73:16-43.
- Thorpe, W. H. 1963. *Learning and Instinct in Animals*. Harvard University Press:Cambridge, MA.

V. CONCLUDING REMARKS

In this dissertation, I have reviewed the literature on learning in rattlesnakes and made recommendations concerning where to begin and how to proceed in studying learning phenomena in rattlesnakes. With learning as a basis, I then attempted to quantify how often western diamondback rattlesnakes use their rattle when encountered by a potential predator. Diamondback rattlesnakes use their rattles much less often than generally assumed. It appears that rattlesnakes use their rattles in just over half of their encounters with humans and presumably other predators as well.

Because rattlesnakes can control when they use their rattle, prior experience must be a major factor in determining when an individual will use its rattle in encounters with potential predators. I performed a laboratory study of habituation of the rattle response in diamondbacks to begin parameterizing the course of habituation. To this end, I designed and constructed an automated apparatus that elicited a rattle response at regular intervals.

Several dependent variables were quantified in search of relevant responses that would exhibit a decrease with repeated stimulation. The occurrence of a response, latency to response, duration of rattling, and the duration of rattling following cessation of the stimulus were all measured. A strict criterion of 10 no response trials followed by a dishabituating stimulus control was implemented to verify habituation.

It was clearly demonstrated in chapter four of this dissertation that experience does influence rattle use. Future studies of learning in rattlesnakes need to apply classical and operant conditioning techniques to tasks involving rattle use. More specifically, it

needs to be verified whether rattling is the result of a simple association between a neutral and unconditioned stimulus or if alpha conditioning better depicts the mechanism by which rattling develops.

For further understanding of the evolutionary origin of the rattle, learning among close relatives of rattlesnakes, such as *Agkistodon* and *Bothrops*, needs to be investigated. Both of these genera exhibit defensive tail vibration in the presence of perceived predators. Experiments should be designed to determine the influence of prior experience on tail vibration in these taxa. If it can be demonstrated that these species modify their use of tail vibration based on prior experience, the evolution of learning can be incorporated into novel hypotheses regarding the evolution of the rattle.

In conclusion, learning is an important aspect of rattlesnake biology that is often over looked by herpetologists, behavioral ecologists, and comparative psychologists. This study is an initial attempt to bridge the gap between these disparate disciplines, but there is much to be discovered about the role learning plays in the lives of rattlesnakes.

VITA

Aaron Jeffrey Place

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Doctor of Philosophy or Other

Thesis: THE ROLE OF LEARNING IN THE EXPRESSION OF RATTLING
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BEHAVIOR IN WESTERN DIAMONDBACK RATTLESNAKES, CROTALUS
ATROX

Pages in Study: 124

Candidate for the Degree of Doctor of Philosophy

Major Field: Zoology

Scope and Method of Study: Field study of rattling behavior in free-ranging western diamondback rattlesnakes. Demonstrated short and long-term habituation of the rattle response in western diamondback rattlesnakes using a new, automated apparatus in the laboratory.

Findings and Conclusions: Rattlesnakes rattle infrequently in the field. Comparisons of free ranging, randomly encountered snakes with free-ranging radio tagged snakes suggest frequent exposure to potential predators modifies rattling behavior. A new, automated apparatus for testing habituation and other learning phenomena related to rattling is described. Habituation of the rattle response does occur over the course of repeated elicitation within several hours and can be evident over several days. Extreme variation in the rates of habituation between individuals was evident.

ADVISER'S APPROVAL: Charles I. Abramson
