Assessing Anxiety in Nonhuman Primates

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Abstract

Anxiety can be broadly described as a psychological state in which normally innocuous environmental stimuli trigger negative emotional expectations. Human anxiety disorders are multidimensional and may be organic or acquired, situational or pervasive. The broad ranging nature of the anxiety phenotype speaks to the need for models that identify its various components and root causes to develop effective clinical treatments. The cross-species comparative approach to modeling anxiety disorders in animals aims to understand mechanisms that both contribute to and modulate anxiety. Nonhuman primate models provide an important bridge from nonprimate model systems because of the complexity of nonhuman primates' biobehavioral capacities and their commonalities with human emotion. The broad goal of this review is to provide an overview of various procedures available to study anxiety in the nonhuman primate, with a focus on the behavioral aspects of anxiety. Commonly used methods covered in this review include assessing animals in their home environment or in response to an ethologically relevant threat, associative conditioning and startle response tests, and cognitive bias tests. We also discuss how these procedures can help veterinarians and researchers care for captive nonhuman primates.

Key Words: anxiety; cognitive bias; human intruder test; macaque; marmoset; startle test

Introduction

Anxiety disorders adversely affect millions of individuals and account for substantial morbidity. The 12-month and lifetime prevalences for anxiety disorders in the United States are greater than 18% and 25%, respectively (Kessler, Berglund, et al. 2005; Kessler, Chiu, et al. 2005), making it the most common mental disorder. Anxiety disrupts an individual's mood and sleep and can negatively affect work and relationships. In addition to its deleterious effect on psychological health and well-being, anxiety can also affect physical health. People with anxiety are at an increased risk for somatic disorders such as cardiovascular disease (Gustad et al. 2013; Scott et al. 2013), hypertension (Jonas et al. 1997; Stein et al. 2014), stroke (Lambiase et al. 2014), irritable bowel syndrome (Gros et al. 2009), and obesity (Brumpton et al. 2012). Anxiety disorders are also a significant societal burden; one study estimated that in 1990, the United States alone spent more than $42 billion on this disease (Greenberg et al. 1999), including treatment and workplace costs (including lost productivity). For all these reasons, anxiety disorders are of great concern.

Although knowledge about anxious behavior and anxiety disorders has increased a great deal in the past several decades, there is still much we do not know. There remains a need for mechanistic studies in an effort to elucidate the physiological underpinnings of anxiety and, in turn, discover more effective treatments, preventative measures, and cures for these disorders. Such mechanistic studies, particularly those that aim to discover the central and peripheral modulators of phenotypic behavioral response, would not only be difficult to perform in humans but would also be ethically prohibited. Thus, animal models will remain a critical component of our investigation of the correlates, causes, and mechanisms that modulate anxiety and other psychological disorders.

Although there are several elegant animal models of anxiety (Griebel and Holmes 2013; Ramos and Mormède 1997; Steimer 2011), the nonhuman primate (NHP) has been increasingly used for several reasons. Unlike rodents, NHPs have an extended lifespan from infancy to adulthood, with definable infant, adolescent, and aged periods. NHPs mature through developmental stages that are known to be landmarks for the onset of psychological disorders in humans (Bennett and Pierre 2010; Forbes and Dahl 2005). Similarly, NHPs have a brain structure comparable in complexity to humans. The NHP brain demonstrates all of the major prefrontal cortical subdivisions, as well as more than two dozen other subdivisions, seen in the human brain (Carmichael and Price 1994; Preuss 1995). Numerous studies have identified both cortical and subcortical structures that contribute to the expression of anxiety-like phenotypes in both NHPs (Amaral 2002; Kalin et al. 2007; Oler et al. 2012) and humans

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(Somerville et al. 2004). Finally, NHPs are physiologically very similar to humans with regard to a number of systems that are often altered in people with anxiety disorders, such as the hypothalamic-pituitary-adrenal axis (Clarke et al. 1995). There is evidence that some physiological measures linked to anxiety in humans, including decreased cerebral spinal fluid levels of the serotonin metabolite, 5-HIAA (Arborelius et al. 1999), are also found in monkeys displaying increased anxious behaviors (Higley et al. 1993; Kalin et al. 2000). Thus, studies with NHPs present unique opportunities to examine the physiologic and neurobiological systems underlying anxious behavior.

These commonalties support the use of the NHP as an appropriate model for understanding various aspects of anxiety and anxiety disorders. However, to perform studies examining anxiety in NHPs, it is necessary to reliably identify signs of anxious behavior in these animals that are analogous to the clinical symptoms used to identify anxiety in humans. Further, it is important to provide convergent lines of evidence demonstrating that the behavioral phenotype(s) are modulated by the same physiologic systems and show similar responses to pharmacologic manipulation. To this end, we will review some of the behavioral methods commonly used to assess anxiety in the NHP, discussing some of the advantages and disadvantages associated with each.

Anxiety and Related Behaviors

Anxiety can be described as apprehension over anticipation of a potential threat. Despite a focus on anxiety disorders, anxiety itself is not inherently harmful. A degree of anxiety in certain situations, such as when confronted with potentially threatening stimuli, is critical to an individual's survival. It is adaptive for an individual to be hypervigilant when predators may be present or for an infant to cry for his or her mother when separated. Indeed, too little anxiety can be potentially dangerous to individuals (Marks and Nesse 1994). However, too much anxiety in situations in which it is not warranted can lead to disruption of normal activities and compromised mental well-being.

In humans, a distinction is often made between an anxious state and trait-like anxiety responses. In general, the anxious state describes a heightened emotional response to a potentially threatening event, such as public speaking. Expected physiologic responses to anxiety-provoking events include activation of the sympathetic nervous system, such as increased heart rate, sweating, and agitation. With repeated exposure to anxiety-provoking events, individuals often adapt strategies that result in a decrease in both the behavioral and physiologic response. Conversely, trait anxiety is often linked to an individual's disposition; individuals with trait anxiety show heightened behavioral response across a larger spectrum of experiences and/or maintain these responses for a longer period of time. This kind of trait-like anxious responding may be associated with anxiety disorders. Thus, anxiety disorders may be thought of as dysregulated biobehavioral responses to novel, unknown, or potentially threatening environmental stimuli. Although distinctions between state anxiety, trait anxiety, and anxiety disorders have been proposed for human populations, there is a great deal of correlation among these concepts (e.g., Mathews 1990). It is difficult to determine whether the same distinctions are present in NHPs. There is no single behavioral response that defines an animal as being anxious. Rather, like humans, NHPs exhibit a continuum of behavioral responses to any given situation. Animals at one end of the spectrum may show heightened or exaggerated responses to a particular stimulus and thus may behave more anxiously than others.

Anxiety in humans is often comorbid with other disorders, including depression and substance or alcohol abuse (Smith and Book 2010). It has also been linked with various temperamental constructs, including excessive fear and behavioral inhibition. Numerous investigations have shown that highly inhibited children are at a greater risk for development of anxiety and depression later in life (Biederman et al. 1993; Hirshfeld et al. 1992; Kagan 2002). Thus, other emotions or traits, in particular fear, are often included in studies of anxiety.

There are many subtypes of anxiety, including separation anxiety, social anxiety, and generalized anxiety, just to name a few. These different subtypes may have evolved to help individuals adapt to various dangers, such as predation, separation from the group, and ostracism from group members (Marks and Nesse 1994). Although these subtypes may manifest themselves differently, they all have many similarities, including the physiologic and behavioral responses and dysregulation of the defense system (Nesse 1999). An in-depth examination into the various subtypes is beyond the scope of this article, and thus we will refer to generalized anxiety unless specifically stated.

Anxiety and the Monkey

Much of the early NHP work on anxiety used macaque species, particularly rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*), as experimental models. These species still remain the most common NHP models of anxiety. However, in the past decade or so there has been a marked increase in the number of New World monkeys, particularly marmoset species (e.g., *Callithrix penicillata*), used in studies of anxiety. Marmosets are smaller and generally easier to handle than macaques and do not have zoonotic concerns such as *Macacine herpesvirus* 1 (Herpes B virus). Although the expression of anxiety includes many species-specific behavioral patterns, the types of behaviors used to identify anxiety are similar across these primate species.

One class of behaviors believed to reflect anxiety in primates, including humans, is displacement behaviors. Displacement activities are typically defined as behaviors that are apparently inappropriate to the stimulus that provoked them. They are thought to occur during situations in which there are conflicting drives (e.g., fear and aggression), unavoidable social or environmental stressors, or in which the

subject cannot attain its goal (Maestripieri et al. 1992). For example, when presented with a food treat from an unfamiliar person, a dog might start licking his paw instead of taking the food. In primates, displacement behaviors can include scratching, auto-grooming, shaking (similar to a wet dog), and yawning. Other signs of anxiety or fear in NHPs include piloerection, or making oneself look larger (Hinde and Rowell 1962). Bared-teeth grins, also known as fear grins or fear grimaces, may indicate fear in various NHP species, although there may be other functions for this facial expression (Petit and Thierry 1992). Increased vigilance and excessive fear are also used to indicate anxiety in NHPs. Slit-stare, flat-tufted ears, and anogentital presentation are anxiety-related displays specific to marmosets (Stevenson and Poole 1976). In addition to facial expressions, vocalizations may also indicate anxiety; young macaques often "coo" when separated from their mother, whereas "tsk-tsk" and "geckering" are indicative of anxiety in marmosets (Stevenson and Poole 1976).

Many of these anxiety-related behaviors are similar to those shown by humans in potentially anxiogenic situations. For example, in humans, displacement behaviors such as scratching or twirling one's hair, similar to self-grooming in NHPs, can indicate anxiety (Troisi 1999). Further, many of these behaviors have been validated by their response upon pharmacologic challenge. Anxiolytics such as lorezapam have been shown to reduce self-directed scratching in adult group-housed cynomolgus macaques (Schino et al. 1991; Schino et al. 1996), whereas anxiogenic compounds increase displacement behaviors (Schino et al. 1996). Thus, these behaviors are generally accepted as indicating anxiety.

Although these specific behaviors are associated with anxiety in NHPs, they are all appropriate in certain contexts. It is when they are performed out of context (e.g., an individual that scratches or shakes in a nonthreatening situation) or for prolonged periods that they may indicate an anxious phenotype. Even adaptive behaviors such as aggression or freezing in the presence of a potential threat can be maladaptive when performed in excess. It is these behaviors that are often of great interest in studies of anxiety.

Assessing Anxiety in NHPs

In humans, clinical or diagnostic information regarding anxious behavior is typically derived by interviews with the patient or caretakers, from administration of standardized inventories, or from direct behavioral assessments. Our ability to assess anxiety in NHP models is much more limited and relies on inferences about behavioral or physiologic outcomes that parallel the human condition. Over the years, researchers have developed various methods to assess specific aspects of anxiety in NHPs in an effort to better understand the necessary or sufficient conditions required to support anxiety-like behavior.

Many of the tests used to assess anxiety in NHPs rely on direct behavioral observations, either in the home environment (in which little is done to the animal) or in a situation in which the animal is somehow provoked (i.e., provided with a stimulus designed to elicit a response). These tests infer that the function of the behavioral responses observed are analogous to those found in people with anxiety. After identifying individuals that express anxious behavior, researchers can then look for concomitant physiologic or neurologic factors that can be translated to the human condition. Alternatively, researchers may also examine ways in which animals with these anxious behaviors differ from others with respect to various outcome measures (e.g., susceptibility to illness, fitness).

Another class of tests relies on objective physiologic or behavioral outcomes to a given stimulus that are homologous to those that occur in humans. For example, anxious people are more likely than others to react to a sudden and unexpected sound with an exaggerated startle response (as measured by heart rate increases or eye blinks). Researchers have adopted similar methodology to assess startle response for NHPs.

More recent assessments of emotionality have focused on cognitive processes involved in emotional regulation. These tests, known collectively as cognitive bias tests, are based on the idea that in humans and other animals, cognitive functions such as judgment and attention can be affected by emotional state. Therefore, one can measure an individual's affective state by assessing his or her judgment about or attention to stimuli with disparate emotional valence. These tests have also been adapted for use in NHPs.

We describe some commonly used methods for assessing anxiety in NHP herein.

Home Environment Assessments

One way in which researchers can evaluate anxiety in NHPs is to observe them in their home environment and assess their response to everyday, naturalistic events (e.g., new caretakers, introduction to novel objects, interactions with conspecifics). Individuals typically respond with a range of behaviors when faced with these sorts of events. Anxious individuals may respond with heightened fear responses compared with other individuals of the same rank, age, and sex. They may also show increased vigilance and displacement behaviors.

To assess behavior in the home environment, researchers typically use standard behavioral techniques (e.g., focal observations, scan observations) (Altmann 1974) to quantify behavior and determine an individual's activity budget. There are several methods by which these sorts of data can be obtained. The most commonly used method is focal observation, in which individuals are observed for a certain amount of time (e.g., 10 minutes a day for several days) and behaviors of interest are recorded. Observations can be taken by a trained observer located in front of the individual or can be videotaped for later behavioral coding. To be effective, these observation periods must be long enough to measure the behavior of interest and frequent enough to ensure that unforeseen events (e.g., illness, change in weather, mechanical problem in room) do not skew the data. Taking shorter,

more frequent observations over several days, as opposed to longer, less frequent observations, allows for a more complete activity budget of the individual.

These types of observational studies have been used to examine social anxiety (e.g., Castles et al. 1999), maternal separation anxiety (e.g., Marais et al. 2006) and generalized anxiety (e.g., response to sudden loud noise; Toxopeus et al. 2005) in NHP species. Although the majority of these tests have been performed on group-housed animals, home environment assessments can be valid for caged and even singly housed animals. In a recent study of singly housed, male cynomolgus macaques (Camus et al. 2013), researchers recorded behaviors (e.g., feeding, stereotypical behavior, behavior toward observer, exploration), location in cage (e.g., front, back), gaze (e.g., toward observer, self), posture (e.g., bipedal, slumped), and body orientation (e.g., toward wall, observer). With a relatively small sample collection (n = 6 observations taken per individual over 2 days), they identified five distinct behavioral profiles. One profile included displacement behaviors and aggression directed toward the observer, which the authors interpreted as analogous to anxious behavior (Camus et al. 2013). Although these home environment behavioral profiles must be validated, they provide evidence that individual differences in the expression of anxious behavior can be obtained with relatively little provocation, even for animals living in cage-housed environments.

Another common method for assessing anxiety and related behaviors in group-housed primates is observer rating (e.g., Capitanio 1999). Rating typically involves two or more observers who score subjects based on a number of predefined traits or adjectives, such as "apprehensive," "active," "playful," and "curious" (e.g., Stevenson-Hinde and Zunz 1978). Scores are then put into a factor analysis, with the goal of revealing various dimensions of behavior. Common factors that typically emerge from such studies include "sociability," "confidence," "fearfulness," "curiosity," and "excitability" (Capitanio et al. 2011; Freeman and Gosling 2010), some of which are linked to anxiety. These factors have been correlated with behavioral responses in various contexts. For example, rhesus macaque infants labeled as having a highly nervous temperament, similar to neuroticism in humans, were more likely than others to develop stereotypical behavior later in life (Vandeleest et al. 2011).

These types of observational studies are highly relevant to anxiety disorders in humans because they highlight the naturally occurring variation in anxious behavior. They also provide an opportunity to examine behavioral responses in situations in which there does not appear to be an overt threat (Schino et al. 1991), which may be analogous with human anxiety. As mentioned above, many of the anxious responses have been validated pharmacologically. However, home environment studies also tend to be highly time intensive and require specialized training in behavioral observation techniques. Sample sizes need to be relatively large to account for factors such as rank and age differences. It is also important to control for other potential confounders, such as time of day, time of year, and so on. NHPs with seasonal breeding/mating patterns may behave differently during the mating season than the birthing season. Even animals without seasonal breeding cycles may behave differently over the course of the year, depending on factors such as length of day and temperature. The time investment and necessary sample sizes are often limiting factors in the effectiveness of home environment assessments as a model for measuring anxiety in NHPs.

Provoked Response Tests

In the past 30 or so years, researchers have used unconditioned responses to various threatening or potentially threatening stimuli in an effort to assess anxiety in NHPs. There are a variety of stressors used in these tests, most of which approximate ethologically relevant stimuli to which the animals should have evolutionary adaptations. These stressors can include a novel environment (i.e., open field tests), novel humans, conspecifics, and predators.

In addition to being ethologically relevant, many of these tests are similar to those used to assess anxiety and related traits (e.g., behavioral inhibition) in humans. For example, one method by which psychologists assess behavioral inhibition and anxiety in children is to measure their behavior in a new environment, often a playroom consisting of novel toys and/or potentially scary stimuli such as masked strangers (Kagan et al. 1988; Pfeifer et al. 2002). The mother is typically present and asked not to interfere with the behavior of the child. Children exhibit a spectrum of responses to this novel environment. Some bolder, more exploratory children play with the toys and show little distress, whereas more inhibited children often stay close to their mothers. Although commonly used to assess anxiety in rodent species (Prut and Belzung 2003), these sorts of open field tests are less often used in NHP species (but see Cagni et al. 2012; Williamson et al. 2003). We focus on two provoked response tests herein.

Human Intruder Test

The human intruder test (HIT) is one of the most widely used tests to measure anxiety in macaques. In this test, the stimulus is an unfamiliar human intruder. The HIT was extrapolated from early studies investigating the expression of species' typical responses to mildly stressful social experiences (Rowell and Hinde 1963) and infant distress responses related to brief maternal separation (McKinney et al. 1972). Kalin and colleagues (e.g., Kalin and Shelton 1989; Kalin and Shelton 2003; Kalin, Shelton, and Takahashi 1991) adopted and refined these early tests to further characterize components of affective responses. In addition, they collected the critical parametric data necessary to put forth an NHP model of anxiety based on individual differences in excessive responses to the human intruder. Throughout the course of these investigations, Kalin and others identified the biobehavioral mechanisms modulating fear, anxiety, and emotive

responses in rhesus macaques and defined the stability of the response across development.

The HIT is designed to measure an individual's response to a potentially threatening social stimulus of an unfamiliar human intruder. The test was originally developed to assess temperament in infant rhesus macaques, although it has been used for animals of all age groups (e.g., Coleman et al. 2011; Corcoran et al. 2012). In the original studies (Kalin and Shelton 1989), the subject was temporarily removed from its mother and moved to a novel cage in a novel testing room. For 10 minutes, the infant remained alone in this room (Alone 1). After 10 minutes, an unfamiliar human intruder entered the room and stood next to the cage, with his or her profile to the subject, taking care to avoid eye contact (No Eye Contact [NEC]). This stimulus was designed to emulate a potential social threat in which the threat does not yet notice the monkey. The human intruder remained in this posture for 9 to 10 minutes, after which the intruder turned his or her head and made direct eye contact with the subject (Stare, ST) for 9 to 10 minutes. Direct eye contact for macaques is a threatening behavior, and this period represented a threatening stimulus for the subjects. The intruder then left the room and the infant was alone for another 10 minutes (Kalin, Shelton and Turner, 1991), after which it was returned to its mother. There have been different iterations of this test; for example, in the original set of studies, the NEC and ST portions were performed on different days (Kalin and Shelton 1989). However, since that original study, the NEC and ST have been performed consecutively, and in some iterations of the test not all four phases of the test are performed.

The premise of the test is to assess response to an ethologically relevant threat stimulus. Although there is a great deal of individual variation in behavioral response on this test, infants tend to respond to the initial separation from their mothers with an increase in coos and locomotion (Kalin and Shelton 1989; Kalin et al. 1998). Coos are a distress vocalization emitted by infants in an effort to attract their mothers (Harlow and Zimmerman 1958). When the human intruder enters without making direct eye contact, the infants often freeze, a behavior in which the subject remains completely motionless except for slight movements of the eyes (Kalin and Shelton 1989). Remaining still and motionless is thought to be an adaptive response for a small defenseless primate by making detection more difficult to a potential predator. Similarly, in the Stare condition, the appropriate adaptive response for a primate would be to vigilant and direct attention directly toward the unknown human stimulus. Infants typically respond to direct eye contact with some degree of defensive behavior such as threats or aggressive vocalizations such as barks (Kalin and Shelton 1989; Kalin, Shelton, and Takahashi 1991).

Although these behavioral responses are adaptive when performed in moderation, they can be problematic when performed to excess, suggesting a dysregulation in what would be considered a normative response to a social challenge (e.g., Kagan 2002). For example, individuals that demonstrate excessive freezing in the NEC may be similar to humans who exhibit excessive or immotile fear responses. There are several lines of evidence suggesting that these individuals indeed exhibit an anxious temperament, validating the translational importance of this paradigm to our understanding of human anxiety (Essex et al. 2009; Kalin et al. 1998).

The establishment of the HIT as a model of anxiety has been further elucidated by pharmacologic manipulation of the neural transmitter systems that are used in the treatment of anxiety disorders. For example, Kalin and Shelton (1989) showed that behavioral responses of infant rhesus macaques to the Alone or NEC conditions were regulated by different neurotransmitter systems. The opiate agonist, morphine, reduced cooing, whereas the opiate antagonist, naloxone, increased cooing, but neither had an effect on freezing or other defensive behaviors. Conversely, diazepam, an anxiolytic benzodiazepine, reduced freezing and defensive behaviors with no effect on cooing. The suppression of these behaviors with anxiolytics establishes their contribution to a phenotypic anxiety-like response. On the other hand, cooing may not be a direct contributor to an anxiety phenotype; rather cooing may be more closely related to the distress response associated with these two test conditions. Subsequent studies have extended the findings of this initial study, showing that anxiolytic drugs decreased both defensive behaviors (Kalin, Shelton, and Turner 1991) and anxiety-related responses to the intruder (Habib et al. 2000) and that an anxiogenic compound increased freezing behavior, exploration, hostility, barking, and cooing (Kalin, Shelton, and Turner, 1992).

Other findings parallel the peripheral measures of anxiety responses found in human studies. In one study, infant rhesus macaques that showed no behavioral response to the human intruder had a blunted growth hormone response to pharmacologic challenge with clonidine and growth hormone releasing hormone compared with infants that did respond, regardless of the nature of the response (Coleman et al. 2003). Blunted growth hormone response to pharmacologic challenge has been found to be associated with the development of depressive (Dahl et al. 2000) and anxious (Abelson et al. 1991) behavior in humans. Similarly, there are numerous studies that probe the dynamics of the hypothalamic-pituitary-adrenal (HPA) axis and the interaction between stress and anxiety-like responses (Arborelius et al. 1999; Owens and Nemeroff 1993). Macaques that showed exaggerated freezing response in the NEC condition (i.e., inhibited response) had higher basal cortisol levels than others (Kalin et al. 1998). This finding is congruent with previous work that demonstrated that inhibited children have higher cortisol levels (Kagan et al. 1988). Thus, blunted growth hormone response or increased cortisol may be indicative of underlying physiologic differences in these systems and their role in modulating affective behavior.

Although the majority of HIT testing has been done with macaques, a variation of this paradigm has been used with marmosets (e.g., Costall et al. 1988). In these tests, a human stranger stands in close proximity to the marmoset in its home cage for a short period of time. Marmosets typically respond to this threat by retreating to the back of the cage and engaging in fear behaviors, including slit stare, scent marking,

flattened ear tufts, and piloerection (e.g., Costall et al. 1988). These behaviors have been reduced with various anxiolytics, including diazepam (Cagni et al. 2009; Carey et al. 1992), buspirone (Costall et al. 1992), zacopride (Costall et al. 1988), and chlordiazepoxide (Walsh et al. 1995).

There are many reasons for the wide use of these human intruder challenge tests. As detailed, they have been well validated, and even slight modifications to the original protocol (e.g., Capitanio 1999; Coleman et al. 2003; Gottlieb and Capitanio 2013; Raper, Wallen, et al. 2013; Raper, Wilson, et al. 2013) have produced similar results. The HIT can be used across development, is relatively easy to perform, and does not require a great deal of specialized equipment.

However, there are some disadvantages of this test as well. Subjects may differ a great deal with respect to their interaction with humans, which could affect their response to an unfamiliar intruder. Subjects living in large groups with little contact with humans may respond differently than subjects living in cages with a great deal of human contact. The consistency of the intruder may also influence of the magnitude of variability of the effects observed. For example, subjects may respond differently to petite female intruders than to large male intruders. Further, in paradigms in which animals are tested in their home cage as opposed to a quiet testing room (e.g., Capitanio 1999), the behavior of the subject can be influenced by the behavior of the other animals in the room.

Predator Confrontation Test

Another ethologically relevant stimulus used to evoke the threat response is a predator. Predation is a driving evolutionary force in most, if not all, primate species (e.g., Barros and Tomaz 2002), and animals have well-developed defensive behaviors to respond to it. Although a human can be a potential threat, defensive behaviors toward actual predators are an intrinsic part of the behavioral repertoire of most animals. Predator confrontation tests (Barros et al. 2000) were developed to assess response to a predatory threat. To date, these tests have been largely performed in marmosets (*Callithrix penicillata*), a species particularly vulnerable to predation in the wild. In these tests, marmosets are exposed to a taxidermized Ocilla cat (*Felis tigrina*), a natural predator. This stuffed predator has been shown to induce consistent threat and fear-related responses in marmosets (Barros and Tomaz 2002).

In this paradigm, the subject is first acclimated to an open-field testing apparatus. The testing space is divided into two main segments with an opaque visual barrier. The larger section is further divided by use of two square holes to basically form a figure 8 maze. A guillotine door in the smaller segment and holes in the barrier allow subjects to move between the segments (Figure 1) (see Barros et al. 2008 for description). At the start of the study, the marmoset is placed in the small section and the guillotine door and other holes in the barrier are removed so that the subject can enter the large section of the testing arena. The marmoset is allowed to acclimate to the maze, without the predatory stimulus, for several (4–7) trials over several days. After the acclimation, the marmoset is again placed in the maze, but with the taxidermized cat present for several trials. The predator is placed in a far corner of the maze in such a way that it is not visible to the marmoset until the subject explores the enclosure (e.g., Barros et al. 2000). This design allows the animal to "happen across" a predator, as it might in a natural environment, as opposed to having a threatening stimulus presented to it while it is in a cage. The design also allows the subject a way to escape the predator (i.e., it can go back to the smaller portion of the enclosure).

As with the human intruder tests, there is a wide range of responses to this paradigm. In general, the marmosets respond to the presence of the stuffed predator with an increase in tsik-tsik alarm calls, vigilance, displacement behaviors such as scratching and scent marking, and a decrease in exploratory behavior, all of which can indicate anxiety (Barros et al. 2000). Researchers found habituation in some variables (e.g., decrease in vocalization and increase in exploration) after prolonged exposure to the predator but not in others (e.g., avoidance of area in which cat was placed) (Barros et al. 2004). There was an increase in self-directed behaviors such as scratching and self-grooming with repeated exposure to the predator (Barros et al. 2004), suggesting these behavioral responses did not attenuate. Administering anxiolytics such as diazepam (Barros et al. 2000) and buspirone (Barros et al. 2001) reduced displacement behaviors and increased proximity to the predator and exploratory behavior in a dose-dependent fashion. Interestingly, as with the HIT, anxiolytics did not affect vocalizations in this paradigm (e.g., Barros et al. 2007).

Similar tests assessing response to a predatory challenge have been performed in other species, including rhesus macaques. Both feral and laboratory-reared rhesus macaques have been introduced to snakes (real and model), a potential predator (Fooden 2000). In these tests (Mineka et al. 1980; Nelson et al. 2003), animals showed fear responses similar to those exhibited by marmosets. As with marmosets, certain behaviors diminished with repeated exposure, particularly with model snakes. Importantly, in both the marmoset and rhesus macaque paradigms, there was a great deal of individual differences in habituation; some subjects acclimated relatively quickly, whereas others did not (Barros et al. 2004; Mineka et al. 1980; Nelson et al. 2003). The lack of habituation, along with heightened fear response, may indicate anxiety in this test.

One advantage of predatory challenge tests over those involving human threats is that they tap into the natural behavior of the subject. Antipredator responses are an important and innate part of the behavioral repertoire of most NHP species. Unlike response to a human, which can be influenced by prior experience, laboratory primates are unlikely to have any experience with a natural predator. However, for these tests to be effective, the predator has to be realistic enough to cause and maintain a response.

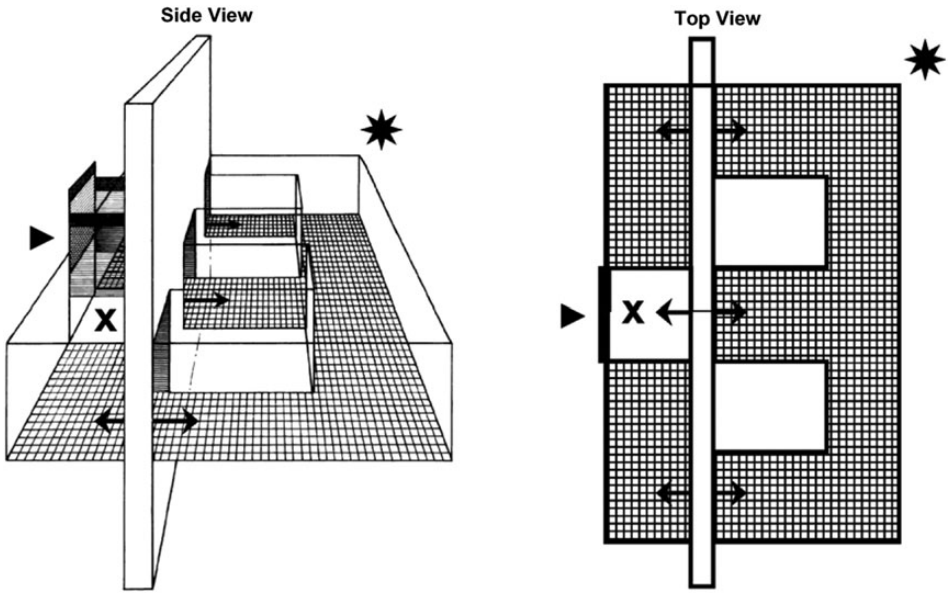

Figure 1 Illustration of the testing arena used in the Marmoset Predator Challenge Test. The asterisk represents the location of the taxidermized predator, and the X represents the starting location for the marmosets. Arrows indicate areas in which the marmoset can move back and forth between the two chambers. Reprinted from Barros M, Alencar C, de Souza Silva MA, Tomaz C. 2008. Changes in experimental conditions alter anti-predator vigilance and sequence predictability in captive marmosets. Behav Processes 77:351–356 with permission from Elsevier.

Associative Conditioning and Startle Response Tests

Associative conditioning models of anxiety, such as the conditioned fear test, use the subject's innate harm avoidance or reflexive escape response to understand the behavioral mechanisms controlling the expression of anxiety. Reflexive startle behaviors in response to an unexpected or noxious stimulus have adaptive value. However, heightened startle responses may indicate anxiety. In this manner, these tests have been used to measure anxiety and related behaviors in many species, including NHPs. In the simplest form of the conditioned fear test, the subject is presented with a neutral or conditional stimulus (CS; e.g., a light) followed by a noxious unconditioned stimulus (US; e.g., a loud noise) known to engender a reflexive unconditioned or startle response (UR; e.g., gross motor movement or eye blink). The magnitude of the animal's motor response (i.e., how much the subject moves; often measured by accelerometer in NHPs) is the primary dependent measure of these associative models, but outcome measures can include other factors such as the duration of the motor response or the occurrence of species-typical threat or anxiety behaviors. Repeated pairings of the CS with the US results in an association between the previously neutral CS and the UR, such that when the CS is presented the animal responds with the UR, even in the absence of the US. In this simplified description, the CS acts to signal the imminent presentation of the startling stimuli (US), which is thought to be fear or anxiety provoking. By investigating the relationship between the presentation of the neutral stimulus (e.g., frequency and duration) and the resulting startle response, researchers can evaluate factors that modulate the subject's expectation of an impending unpleasant stimulus. In this manner, associative models provide a framework for understanding how previously paired associations between neutral and threatening stimuli come to engender negative emotive responses (e.g., fear) and trigger an expectation of the noxious stimulus (i.e., an anxious state). Exaggerated emotional responses to seemingly unthreatening stimuli are a hallmark of anxiety disorders and can be behaviorally expressed as either hyper- or hyporesponsiveness to these environmental stimuli (Kagan 2002).

Variations of the conditioned fear test and other associative models have further allowed scientists to investigate the contributions of these paired associations to the expression of anxiety. One variation, the potentiated startle test, uses the CS (e.g., light cue) previously conditioned to signal an aversive stimulus (US; loud noise in the previous example) to intermittently signal a second aversive stimulus (e.g., an air puff). The manipulation of intermittent presentation of the CS results in a potentiated behavioral response. A heightened startle response (i.e., increased magnitude and/or duration) to the CS correlates with anxiety or fear (for review, see Davis 1986). Potentiated startle has been widely validated in a number of species, including various NHPs (see Winslow et al. 2002). The correspondence between human and NHP responses allow for further investigation of the mechanisms that modulate these responses. For example, potentiated startle response can be blunted by the administration of anxiolytic compounds and augmented by drugs with known anxiogenic properties (Davis 1986).

The prepulse inhibition (PPI) test is similar to the conditioned fear test. The PPI test first establishes the CS–US pairing but then adds another cue or prepulse stimulus (e.g., weak puff of air) just before presentation of the aversive stimulus. The prepulse stimulus typically reduces or inhibits the magnitude of behavioral response (i.e., gross motor output) to the aversive stimulus, although there is individual variation in the amount of inhibition. This model assesses the subject's ability to use the additional sensory information from the prepulse stimulus to gate the effects of an imminent aversive stimulus. This sensorimotor gating is attenuated in various psychopathologies, including schizophrenia, which is often comorbid with anxiety (Morris et al. 2010). More recently, decreased PPI has been shown in humans with panic disorder (Ludewig et al. 2002) and general anxiety (Duley et al. 2007). Although few studies have explicitly used the PPI test as a model for anxiety in NHPs, PPI has been found in rhesus macaques (Winslow et al. 2002), allowing for studies examining contributions of neural systems in modulating anxiety in NHPs (e.g., Davis et al. 2008).

Finally, although not a model of associative conditioning, the unsignaled acoustic startle test has also been used to assess emotionality in NHPs (Winslow et al. 2002). In this test, the subject is typically put in a specialized chamber and presented with a nonsignaled, uncued startle stimulus (e.g., acoustic stimulus). In NHPs, the magnitude of the response is measured as gross motor movement or defensive behavioral reactions such as increased vigilance and open mouth threat. Subjects typically begin to adapt to the startle stimulus over repeated presentations, and the behavioral response diminishes. However, there is a continuum of individual differences in learning to habituate to the response. Subjects that continue to exhibit a heightened or prolonged startle response after repeated trials are conceptualized to be "anxiety sensitive" (McMillan et al. 2012; Winslow et al. 2002). Similarly, Ludewig and colleagues (2002) found that human subjects with panic disorder showed a normal startle response but had deficits in ability to habituate to unsignaled startle.

All of these tests are designed to provide objective behavioral measures of emotionality (Winslow et al. 2002). The behavioral responses are reflexive, not inferred, as they are with some of the other tests mentioned. For example, gross motor movement, often assessed by the use of an accelerometer, is a common outcome measure for these tests. Associative tests also require a relatively small number of subjects and produce reliable and repeatable cross-species comparisons from NHP to human. Similarly, measuring the responsiveness of the fear conditioning tests described herein to pharmacologic manipulation with anxiolytic drugs has also informed our understanding of the systems that mediate anxiety responses. Benzodiazepines are known to be effective in treatment of anxiety reactions, and they also modulate performance of these tasks in a manner that decreases potentiated startle amplitude (Graham 2005; Winslow et al. 2007) and acquisition of acoustic startle responding (Scaife 2005).

There are considerations that need to be made when choosing these types of tests. The responses of humans with anxiety disorders on the conditioning and startle response tests do not always directly parallel those in NHP and other animal models. This may reflect the different contexts in which these tests are performed, species differences, and the numerous subtypes of anxiety disorders (see Grillon 2002). Further, these models require a substantial financial cost for equipment, training, and professional expertise in animal handling and behavioral analysis. Finally, these tests require a large initial time investment compared with other tests because animals may need to be trained to be removed from the housing environment and relocated to and from a testing environment. Still, the condition fear tests outlined herein continue to help refine and inform our understanding of the biobehavioral mechanisms that support anxiety-like behavioral responses.

Cognitive Bias Testing

One interesting new method of assessing emotional states, including anxiety, in animals is cognitive bias testing. As with the associative testing, it does not focus on inferring fear or anxiety-related behaviors. Rather, it assesses aspects of cognitive processes, such as judgment, attention, and memory (e.g., Mendl et al. 2009; Paul et al. 2005). Studies in humans have shown that an individual's emotional state affects these processes (e.g., Vuilleumier 2005). People in an anxious or otherwise negative emotional state are more likely than nonanxious control subjects to interpret ambiguous stimuli as threatening (e.g., Eysenck et al. 1991; Mathews et al. 1997) and tend to spend longer attending to negative stimuli than neutral or positive stimuli (Bar-Haim et al. 2007; MacLeod et al. 1986; Mineka and Sutton 1992; Mogg and Bradley 1998). Increased vigilance in a potentially threatening environment can have adaptive value because it allows individuals to appropriately respond to potential threats. However, when taken to an extreme level, these behavior patterns can be maladaptive. While there are a number of theories surrounding the etiology of anxiety, it is generally believed to be associated with enhanced sensitivity toward threat-related stimuli (Bar-Haim et al. 2007). By measuring bias toward negative and potentially threatening stimuli, these tests evaluate the individual's sensitivity towards threat. To date, the majority of these tests have not assessed anxiety per se (however, see Lacruese et al. 2010), and few have been validated pharmacologically (however, see Doyle et al. 2011). Still, by measuring the individual's sensitivity toward threat, these tests have the potential to assess anxiety in NHPs.

In humans, cognitive bias tests assess judgment, attention, and memory. To date, the vast majority of tests used to assess emotionality in nonhuman animals have focused on judgment and, to a lesser extent, attention (however, see Paul et al. 2005 for discussion on emotion and memory). We will therefore focus this discussion on those two aspects of cognitive bias.

Mendl and colleagues (Harding et al. 2004) are credited with designing the first cognitive bias testing for animals. This pioneering study used a judgment bias test in which subjects were effectively asked to judge a neutral cue. Although there are many versions of judgment bias testing (see Mendl et al. 2009 for comprehensive review), in general, subjects are trained to pair specific cues (e.g., blue vs. red color) with specific behaviors (e.g., touching a square vs. triangle) to achieve various outcomes. The outcomes are typically either positive and negative (e.g., food treat vs. mild shock) or positive and less positive (e.g., food treat vs. no food or low-value food treat). Subjects are then presented with a neutral cue, which is in between the two established cues (e.g., purple color). For example, subjects may be trained to touch a square paired with a blue light to get a large reward and to touch a triangle paired with a red light to get a small reward. In this scenario, subjects get no reward for touching the square when it is paired with the red light or for touching the triangle when paired with the blue light. Subjects are then presented with both the square and triangle paired with a purple (intermediate) light. How animals respond to this ambiguous cue is thought to be indicative of their emotional state; animals in a positive emotional state should be more likely to interpret the neutral cue as leading to the positive outcome (and thus respond optimistically), whereas those in a negative state should be more likely to interpret the neutral cue as leading to the negative (or less positive) outcome, and thus respond pessimistically. In the example above, an optimistic response to the purple light would be to touch the square.

Since Harding's initial study, there has been a great deal of interest in judgment bias testing. It has been used to assess emotionality in a variety of species, including rats (Brydges et al. 2011; Harding et al. 2004; Richter et al. 2012), capuchins (Pomerantz et al. 2012), and rhesus macaques (Bethell et al. 2012a). Many of these tests examined bias when animals were exposed to a positive event (e.g., provision of enrichment) and a negative event (e.g., lack of enrichment or after a stressful procedure such as a health exam). As expected, the majority of studies found that animals responded more optimistically during the positive event than the negative event.

Recent studies have used judgment bias tests to assess emotionality in subjects with different temperaments. Mendl and colleagues (2010) used this test to assess bias in dogs with and without separation anxiety. Subjects were exposed to food bowls placed in two locations. The bowl in the positive location always had food, whereas the bowl in the negative location was always empty. The dogs quickly learned this association and approached the bowl in the positive location more quickly than the one in the negative location. However, when presented with a bowl in a neutral location (halfway between the positive and negative locations), dogs with separation anxiety had a significantly longer latency to approach the bowl compared with dogs without separation anxiety, suggesting that they had a more pessimistic view of the reward (i.e., associated the neutral placement with the negative, as opposed to positive, location) (Mendl et al. 2010). Other studies have examined the correlation between cognitive bias and the presence of stereotypical behavior. Stereotypies are repetitive, habitual behavior patterns with no obvious goal (Mason 1991; Shepherdson 1993) and are often considered to indicate compromised well-being (Mason 1991). Capuchins with high levels of head twirls, a specific pattern of stereotypy characterized by circular movements of the head, were more likely than animals with low levels of this behavior to respond pessimistically to ambiguous cues (Pomerantz et al. 2012). These studies suggest promise for the use of cognitive bias tests to assess emotional states in animals. Subjects across species with behavioral indications of stress and/or anxiety have been shown to behave pessimistically on judgment tests.

Cognitive bias tests based on attention have also been used to measure emotional state in humans and other animals (e.g., Bar-Haim et al. 2007). People with anxiety are more likely to attend to negative stimuli or words than nonanxious control subjects. The dot-probe task is often used to assess attention to threatening stimuli in humans. In this test, the subject is briefly presented with two images with different emotional valence (e.g., angry and happy faces). After the images are removed, a dot appears in place of one of the images and the subject is asked to note its location. Anxious people are quicker to identify the location of the dot when it was in the location of the threatening stimulus compared with the location of the neutral or positive stimulus (Bar-Haim et al. 2007; Mogg and Bradley 1998).

Despite its use in human studies of anxiety, there is a paucity of attention bias tests in animal studies. However, a modified version of the dot-probe task has been used in rhesus macaques (e.g., King et al. 2012; Lacreuse et al. 2010; Lacreuse et al. 2013). Lacreuse and colleagues (2013) found that, like humans, male macaques were more likely to attend to threatening, as opposed to neutral, conspecific faces. Using a different test to measure attention, Bethell and colleagues (2012b) assessed emotional state of rhesus macaques after either a negative husbandry event (health exam the previous day) or a positive event (provision of enrichment). Monkeys were exposed to two computer monitors displaying either a threatening or neutral conspecific face. Subjects looked at the aggressive faces more quickly than the neutral faces, regardless of whether they had undergone a health exam or were in the enriched condition. Animals disengaged from the aggressive faces more quickly and spent less time attending to the aggressive faces after having undergone the health exams than during enrichment, which the authors interpret as evidence of emotion-mediated avoidance of threatening faces (Bethell et al. 2012b). Although this result differs from their hypothesis, which stated that individuals in an anxious state would spend more time attending to threat, studies have shown that human patients with high levels of social anxiety spend less time attending to emotional, compared with neutral, faces (Garner et al. 2006). These findings suggest that these sorts of attention bias tests may be useful tools for assessing anxiety in NHPs.

Although cognitive bias tests hold great promise, there remain some unresolved issues (see Mendl et al. 2009 for

comprehensive overview). Motivation for a food reward can affect how animals behave on the task independent of affective state. General animal activity can also affect response, particularly for studies in which the animals are asked to either press or not press a lever for reward (i.e., "go, no go" tasks). Furthermore, many of these tests require intensive animal training. Not only can this training take a significant amount of time, there can also be vast differences in how long it takes to train various animals for specific tasks. This discrepancy could be due, at least in part, to the animal's temperament. Highly inhibited and fearful rhesus macaques are harder to train for simple tasks compared with more exploratory animals (Coleman et al. 2005). Thus, animals excluded from the test because they cannot be trained may be the ones that are of interest to these studies (i.e., anxious individuals). In other words, studies involving training could bias the subject pool. Because of this bias, tests that can be performed without intensive training, such as those that focus on attention, might be preferable to those that require such training, at least in certain cases.

In addition, compared with some other tests of anxiety, there have been few studies that have validated these kinds of tests, either pharmacologically or behaviorally. In one of the few, Doyle and colleagues (2011) found that administering p-chlorophenylalanine, a serotonin inhibitor, to sheep for 5 days increased their pessimism on a judgment bias test and that pessimistic responses correlated with an increase in cortisol. More research is needed to validate these cognitive bias tests.

One potential advantage of these kinds of tests over others is that they rely on the correlation of cognition and information processing as opposed to behavioral responses. In other tests, behavioral responses need to be interpreted, and that interpretation may not always be consistent across studies. For example, yawning in rhesus macaques has been used as a measure of anxiety by several authors (e.g., Machado et al. 2009) but has also been interpreted as mild aggression by other authors (e.g., Meunier and Bachevalier 2002). Cognitive bias testing avoids these kinds of interpretation issues. In addition, cognitive bias tests performed on animals are very similar to those used in humans, thus providing direct translational value.

Anxiety Assessments and Care of Captive Primates

The tests mentioned in this review have great value in translational studies of human anxiety disorders. However, the ability to reliably assess anxiety in captive NHPs can also be of value in management practices. Because of the stoic nature of NHPs, interpreting their emotional state can be difficult. The same tests used to measure anxiety in captive NHPs for research reasons can be used to assess anxiety for management and/or veterinary care purposes.

There are many practical benefits to evaluating behavioral characteristics, including anxiety, in captive animals. Anxiety is associated with the development of clinical and behavioral problems in humans and other animals. Children who are behaviorally inhibited, a trait closely related to anxiety, are at a greater risk for developing diseases such as asthma (Ortega et al. 2002) and other respiratory illnesses (Boyce et al. 1995). Similarly, behaviorally inhibited rhesus macaque infants are more likely than others to exhibit airway hyperresponsiveness, a characteristic of certain kinds of asthma, later in life (Capitanio et al. 2011). There are behavioral concomitants to anxiety as well. As mentioned herein, inhibited children are at a greater risk than others for developing psychopathologies, including anxiety disorders and depression, particularly in response to a major life event such as death of a parent (Hirshfeld et al. 1992; Schwartz et al. 1999). Inhibited or anxious NHP infants may have attenuated behavioral responses to stressful events as well. Infant rhesus macaques that responded to the HIT with behavioral inhibition when tested at 3 months of age were more likely than others to show distress behaviors (e.g., anorexia) after weaning from their mothers at 6 to 8 months of age (K. Coleman, unpublished data). Further, there is evidence suggesting that anxious NHPs may be more likely to develop stereotypic (Vandeleest et al. 2011) or self-injurious behavior (Major et al. 2009). Thus, behavioral response on tests such as the HIT can help identify individuals that may be predisposed to develop clinical or behavioral problems.

Anxiety can also help predict how animals will handle various stresses, including those associated with husbandry practices or scientific protocols. For example, highly stress-sensitive or anxious cynomolgus macaques are more likely than others to develop amenorrhea in response to moving to a new room, a relatively common stress in research facilities (Cameron 1997). Similarly, Capitanio (2010) found that rhesus macaques with temperamental construct of low sociability (similar to social anxiety) exhibited altered regulation of the HPA axis and altered immune function when they were in an unstable social environment (i.e., one in which group members changed regularly). Thus, even common husbandry practices, such as moving animals, may negatively impact anxious individuals more than others. This information can be used to reduce the impact of the stressful event on individuals. For example, at many facilities, primates known to be anxious or stress sensitive are provided with additional enrichment before the occurrence of stressful events such as cage changing. Information about anxiety can also be used to inform subject selection for research studies. Highly anxious or inhibited monkeys may not do well in studies that involve a great deal of change or stress. Even events not generally considered stressful can provoke an untoward response in anxious individuals. Yamanashi and Matsuzawa (2010) examined the behavior of six chimpanzees while they were performing various cognitive tasks. Half of the chimpanzees responded to these tasks with an increase in self-directed behaviors such as scratching. These anxious chimpanzees were more likely than others to become agitated when they got a wrong response.

Finally, individual differences in response to anxiety tests can affect behavioral management practices such as

socialization and positive reinforcement training (Coleman 2012). With the new edition of the *Guide to the Care and Use of Laboratory Animals* (National Research Council 2011), there has been an increased emphasis on social housing laboratory primates. However, social housing can result in aggression and injury if the partners are not compatible. There is evidence that rhesus macaques are more likely to affiliate with conspecifics having a similar, rather than dissimilar, temperament, as measured either by the HIT (McMillan et al. 2003) or home environment assessments (Weinstein and Capitanio 2008). Anxious individuals may be more compatible with similarly anxious individuals. Positive reinforcement training (PRT) is another component of behavioral management. PRT is a type of training in which the subject gets rewarded for performing certain behaviors. Because PRT provides animals with a sense of control over their environment, it is generally considered to reduce stress (e.g., Laule et al. 2003). Not every subject may benefit equally from PRT, however. In one study (Coleman et al. 2005), inhibited and exploratory rhesus macaques (as assessed by the HIT) were trained to touch a target placed on the outside of their cage, a simple task. The inhibited animals were less likely than others to reliably touch the target. Further, trainers noted that the inhibited monkeys often stayed in the back corner of the cage during the training sessions. Thus, for these anxious animals, training may not have provided the same psychologic well-being benefits afforded to other individuals. Instead, training may actually increase stress for these animals.

Knowing the specific behavioral characteristics of captive NHPs can help veterinarians and others attend to their unique behavioral needs. This kind of personalized care will, in turn, help promote the welfare of the animals.

Conclusions

Anxiety is a serious condition requiring a great deal of study. There are many animal models of anxiety and several different kinds of tests, including home cage testing, fear conditioned response, provocative tests, and cognitive bias testing. We touched on some of the commonly used tests in this review, but there are many others, each with advantages and disadvantages. Studying animals in their natural environment allows one to assess anxiety in an unprovoked state but can take a great deal of resources to accomplish and is not highly controlled. Provoked tests, in which the subject is confronted with a potentially threatening stimulus, are highly controllable and can be effective, but observed results may be limited by the stimulus. In other words, exposing animals to a social threat may not indicate how they would respond to a nonsocial threat. Associative tests are highly reliable but are somewhat more invasive than other tests and require specialized equipment. Cognitive bias testing is still in its infancy but shows great promise.

It is likely that no single test will capture all elements of anxiety. With the exception of cognitive bias testing, each of these tests measures a specific aspect of anxiety, whether it is in a social situation or response to a novel object or potential predator. However, how animals respond to one stimulus does not necessarily predict how they will respond to other stimuli; responses can be specific to the context in which they are tested (Carter et al. 2012; Coleman and Wilson 1998). For example, Williamson and colleagues (2003) assessed exploratory behavior in infant rhesus macaques (aged 3–6 months) using three different testing paradigms—the HIT; a free play test, in which the infants were exposed to novel toys in the presence of their mother; and a novel object test, in which the infants were exposed to novel food items while in a cage by themselves. Propensity to explore in the three tests loaded onto different factors in factor analysis (Williamson et al. 2003). Further, behavior in one test did not correlate with the others, suggesting that responses were context dependent. This finding is not necessarily surprising; people with a great deal of social anxiety can show little anxiety in other parts of their lives (e.g., shy individuals may engage in risky behaviors such as skydiving). Thus, to get a more complete understanding of anxiety and other related behaviors, a multitude of tests may be appropriate.

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