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The Human Intruder Test: An Anxiety Assessment in Rhesus Macaques (Macaca Mulatta)

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THE HUMAN INTRUDER TEST:
AN ANXIETY ASSESSMENT IN RHESUS MACAQUES (*MACACA MULATTA*)

A Thesis Presented

by

EMILY J. PETERSON

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DEDICATION

To my parents for their endless encouragement
To Colin for his patience and love

&

To all animals
For teaching me to love behavior

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ABSTRACT

THE HUMAN INTRUDER TEST:

AN ANXIETY ASSESSMENT IN RHESUS MACAQUES (*MACACA MULATTA*)

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The human intruder test (HIT) is a noninvasive tool widely used for assessing anxiety in rhesus macaques (*Macaca mulatta*). This thesis explores the HIT procedure and applies it to a population of monkeys with a self-injurious behavioral pathology. Individual variation on this test can be used to assess anxiety and temperament. The first experiment of this thesis applied two different procedures of the HIT to 17 monkeys at UMass. Monkeys displayed little response to the intruder, and no significant differences were detected for the two procedures. To determine whether these responses were unique to the UMass monkeys, their behavior was then compared to the behavior of monkeys at three other primate facilities. UMass monkeys showed less of a reaction compared to monkeys at other facilities. They came to the front of the cage when the intruder entered the room whereas the monkeys at other facilities moved to the back and showed virtually no threats to the intruder. One possible explanation is the increased exposure to humans that UMass monkeys experience. Even though the human running the HIT was a stranger, monkeys at UMass may not perceive a new human in front of their cage to be a threat. The second experiment tested the hypothesis that monkeys with a record of self-injurious behavior (SIB) would be more anxious in response to the HIT. The cage-side version of the HIT was applied to 41 monkeys with a record of self-injurious behavior and 36 matched controls. In contrast to our prediction, SIB subjects spent significantly less time showing anxious behavior and aggressive behavior toward the intruder as well as spent more time in the front of the cage. SIB subjects showed the same range of behaviors as controls, but significantly less behavioral change overall. These data add to the evidence from experiment one that the HIT may not be a sufficient novelty test to elicit a response in monkeys who are more often exposed to different people. An alternative explanation is that SIB is associated with a depressive like syndrome based on reduced overall activity and possibly lowered affect during the stare phase.

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CHAPTER 1

INTRODUCTION

1.1 The Human Intruder Test

The human intruder test (HIT) is a provoked response test widely used to identify an anxiety-like endophenotype in laboratory rhesus macaques (*Macaca mulatta*) [Kalin & Shelton, 1989; Coleman & Pierre, 2014]. Emulating a stranger test used with human children, Kalin and Shelton developed the first HIT in the late 80s to assess defensive behaviors of infant macaques [Kalin & Shelton, 1989]. By exposing infant macaques to the novel social situation of a stranger and evaluating the behavioral responses to this ecologically relevant threat, Kalin and Shelton were able to categorize different infant macaque coping strategies. Based on the intensity of the response phenotypic groups can be determined. These phenotypic groups can then be used to improve welfare or gain more information about each group.

Though utilized extensively with infant monkeys, fewer studies have used the HIT with adults. The original paradigm involves removing the infant monkey from its mother, placing it in a testing cage, and exposing it to an unfamiliar experimenter. The infant is exposed to two experimenter phases: a no eye contact phase (with the intruder in a profile orientation) and a direct eye contact phase (intruder facing directly toward the subject), with phases before, after, and in-between the intruder phases in which the monkey is alone [Kalin & Shelton, 1989]. This test includes the traditional phases: alone, profile, and stare commonly used in subsequent HITs. Adult monkeys show a different behavioral repertoire than infant monkeys during the HIT. Infant monkeys often respond to the test with cooing or barking vocalizations whereas adult monkeys' responses generally involve little to no vocalizations. For all ages the human intruder test provokes a response that can be used as a metric to assess defensive reactions and coping strategies [Corcoran et al., 2012].

In a laboratory setting the HIT is a useful non-invasive tool to assess anxiety in adult macaques. Because the test poses only a mildly threatening stimulus a range of reactivity is seen. With enough subject variability in the reactivity to the intruder, subjects can be divided into high and low reactors. The highly reactive monkeys may be considered to have an anxious-like phenotype when compared with the low reactors. Further research on anxiety can be then conducted on this group of anxious animals. Variations of the original procedure make the test more applicable in large facilities as well as in a variety of housing conditions. These variations include differences in the length of the phases, order of phases, positioning of the intruder (distance from the cage), and location of the test subject (novel room or home cage).

Kalin and Shelton assessed how the mothers reacted to the test when separated from their infants; this was the first time the test was used on adult macaques. Cortisol, a commonly used biomarker for stress, was extracted from blood plasma in this study. The adult female was removed from the cage containing its infant and placed in a novel testing cage. This study used a two phase test in which the first phase was a 10-minute alone phase and the second was a 10-minute no eye contact phase (profile orientation standing 2.5 meters from the cage). The no eye contact phase and the subject's cortisol levels were positively correlated with the time they spent freezing [Kalin et al., 1998]. This study demonstrated that the HIT elicits a reaction from adult macaques in addition to infants, even with a very restricted design.

A variation of the HIT with multiple intruder distances and shorter phase lengths was used when investigating personality dimensions in adult male macaques. Four personality factors were defined (Sociability, Confidence, Excitability, and Equability) by observer ratings of baseline behavior using twenty-five adjectives. Monkeys were tested on the HIT to determine if responsiveness to an intruder was related to any of these dimensions. Monkeys stayed in their home-cage for this test and were tested five days in a row. This test only had intruder phases and each phase was 30 seconds. The four phases, which were run consecutively in a random order, were profile orientation in close proximity to the cage and also at a distance from the cage (near vs. far), and stare orientation near the cage and far from the cage. The two distances were 0.3 and 0.8 meters respectively. Profile/far was considered to be the least evocative phase with the stare/near considered the most evocative phase. Behavioral differences during these two phases were compared. The excitable personality trait was positively correlated with the increase in threatening behavior and negatively correlated with the time spent in the back of the cage during the stare phase (near condition). None of the other personality dimensions showed any relationship to the test [Capitanio, 1999]. Threatening and time spent in the back of the cage are behaviors often scored when analyzing a monkey's response to an intruder. It is perhaps not surprising that excitable monkeys would react more aggressively to the intruder by threatening and spending more time in the front of the cage to confront the intruder. An alternative strategy is to freeze and spend more time in the back of the cage. However, none of the personality dimensions examined in this study involved a behaviorally inhibited personality. It is possible that there may be two distinct defensive strategies. Retreating to the back of the cage in an attempt to escape from the intruder or, as seen in more excitable monkeys, approaching the intruder to threaten in an offensive manner. In this study, short phases presented in a random order were used to prevent habituation to the intruder in this test. However, there was no mention of how the monkeys' behavior changed over the five consecutive trials.

Based on this multi-distance variation of the test, a similar variation of the HIT was utilized to examine the responsiveness of adult monkeys with Klüver-Bucy syndrome, a syndrome known to cause changes in responsiveness to novel stimuli resulting from bilateral lesions in the amygdala. Two intruder conditions were used in this test. The technician acting as an intruder for this test was familiar to the subject (familiar condition) and wore a cap, glasses, and a mask with lips to create a stranger condition. Four 15-second phases: profile and stare orientations at two distances (near the cage at 0.5 meters and far from the cage at 1 meter), were run on monkeys with both familiar and stranger conditions. All eight phases were run while they were in a familiar testing cage out of view from conspecifics. The order of conditions was randomized each day, and six days in a row the HIT was run with a food-offering task directly following the four intruder positions in each condition. Monkeys with lesions showed decreased inhibition in response to the intruder regardless of distance or orientation, spending more time in the front of the cage compared to controls. The familiarity conditions did not significantly affect the monkeys' responses. The controls showed acclimation to the test by the last three testing days, demonstrating a decreased reaction to the intruder and similar behavior to the amygdala lesion subjects in the last three days tested [Mason et al., 2006]. This study not only demonstrates a lack of inhibition in monkeys with Klüver-Bucy syndrome, but also provides evidence for habituation to the intruder over time.

Habituation to the intruder may be reduced if the intruder phases are separated by baseline (alone) phases. A version of the HIT with the alone phases was used to assess the anxiety of ovariectomized female macaques. Corral housed subjects were brought inside to a testing cage and had a twelve minute acclimation period before an unfamiliar person entered the room. Four 2-minute phases followed this acclimation period: intruder in profile, subject alone, intruder staring, followed by another subject alone phase. The intruder stood 0.3 meters from the cage in both the profile and stare phases. This test was run once per subject. Ovariectomized females tended to freeze more than controls throughout the test, however this was only significant during the second alone condition, where controls did not continue to freeze but ovariectomized females did. The authors discussed the small sample size and large individual variation as a limitation of this study [Coleman et al., 2011].

A different variation of the alone phases was adapted as part of a HIT used to assess reactivity of adult male macaques from different rearing conditions. The HIT consisted of a 10-minute alone baseline phase, a profile phase where the intruder sat 2.5 meters from the cage, a three-minute alone phase in which data were not collected, a 10-minute intruder stare phase (2.5 meters distance), and a final 10-minute baseline phase. During the alone phases the monkeys spent more time in locomotion and facing forward than in the intruder phases. The monkeys froze significantly more during the profile phase than the two alone phases and lipsmacking occurred during the stare phase more than any other phase. Overall, nursery-reared monkeys were more inhibited when compared to mother-reared. Nursery-reared monkeys showed less locomotion and exploration across all phases of the test and exhibited more freezing behavior [Corcoran et. al., 2012].

To generate a cage-side variant that can be easily run at large research facilities, Kristine Coleman refined the original HIT protocol [Coleman, 2015 in press]. The alone phases were eliminated, and a third intruder phase, involving the experimenter facing away from the subject, was added to the end of the test to assess the significance of seeing the face of the intruder. The cage-side variant was run in the colony room thereby eliminating the need to remove the subject from their home cage. This simplified the test and removed the confounding factor of transport stress.

Although many variations of the HIT have been run, many of these variations have similarities. As the main core of the test, all HITs have an intruder phase, with variations in distance and orientation. Commonly a baseline phase is used and alone phases between multiple intruder phases have also seen in more recent variations. Phase length varies between variations of the test as well. A summary of the variations of the HIT discussed is shown in table 1.

Table 1.1 Variations of the HIT used in adult macaques

Phases:	Alone	Profile	Alone	Stare	Alone	Back
Kalin et al., 1998	10 mins	10 mins	X	X	X	X
Capitanio, 1999	X	Near 30 s --------- Far 30 s	X	Near 30 s --------- Far 30 s	X	X
Mason et al. 2006+	X	Near 15 s --------- Far 15 s	X	Near 15 s --------- Far 15 s	X	X
Coleman et al., 2011	12 mins	2 mins	2 mins	2 mins	2 mins	X
Corcoran et al., 2012	10 mins	10 mins	break	10 mins	10 mins	X
Cage-side*	10 mins	2 mins	X	2 mins	X	2 mins
Traditional*	2 mins	2 mins	2 mins	2 mins	2 mins	2 mins

+ Both familiar and stranger conditions used for all phases
* Tests run for this study, only last two minutes of baseline behavior scored
X this phase was not included in the test

1.2 Anxiety

Rhesus macaques are well-established models to study anxiety and emotional regulation in humans [Kalin & Shelton, 2003], and the HIT has become one of many paradigms used to assess reactivity, as a proxy for anxiety, in this model. Physiological studies show analogous brain regions and physiological systems associated with anxiety and emotional affect in humans and monkeys. The orbitofrontal cortex and amygdala are involved in anxiety and affective responses [Kalin et. al., 2007] in both species. Additionally, the hypothalamic-pituitary-adrenal (HPA) axis is also well studied in both species in relation to anxiety-like disorders [Arborelius et. al., 1999]. Anxious behavior includes exaggerated or inappropriate responses to a threatening situation that include increased rates of scratching, yawning, and self-grooming. Additionally, behavioral inhibition (a restriction of typical behavior patterns and expressions) has been identified as a sign of anxiety in both infant macaques and children [Kalin & Shelton, 2003]. Fear responses to a threat (such as fear grimacing or freezing in macaques) may be over exaggerated in anxious individuals [Kalin & Shelton, 2003; Coleman & Pierre, 2014]. Some of these behaviors have been physiologically linked with anxiety through pharmaceutical studies. The rates of scratching and yawning can be decreased with the anxiolytic drug lorazepam and increased following exposure to the anxiogenic drug FG7142 [Schino et al., 1991 & 1996]. Anxiety may also play a role in the development of abnormal behaviors such as self-injurious behavior but this linkage has not been fully explored in the monkey model.

1.3 Self-Injurious Behavior

Self-injurious behavior (SIB) is a serious human health concern that has been observed and studied in both human and nonhuman primates. In rhesus monkeys, SIB is usually manifested as self-directed biting that may lead to wounds requiring veterinary care [Novak, 2003]. In some laboratory facilities this pathology can be seen in up to 25% of the population [Lutz et al., 2003]. There are concordances between SIB in rhesus macaques and the non-suicidal self-injury (NSSI) reported in the general human population [see Novak et al., 2014 for a review]. Similarities include similar targets for injury, albeit using different means (i.e., to arms and legs), presence of early life stress events [Lutz et al., 2003], and somewhat similar responses to treatment, e.g., some efficacy with fluoxetine and naltrexone [Gottlieb et al., 2013].

A variety of environmental factors and physiological correlates have been explored to gain a better understanding of this behavioral pathology in monkeys [Novak, 2003; Novak et al., 2014]. Restrictive environments and atypical rearing conditions are associated with the development of SIB in monkeys [Kraemer et al., 1997; Bellanca & Crockett, 2002; and Lutz et al., 2003]. For example, nursery-reared monkeys are more likely to develop SIB than mother-reared monkeys [Lutz et al., 2004; Gottlieb et al., 2013]. The stress system has been explored in monkeys with SIB. Monkeys with SIB appear to have a blunted hypothalamic-pituitary-adrenal (HPA) axis, as manifested by an attenuated response to stimulation by adrenocorticotrophic hormone (ACTH) compared to control monkeys [Tiefenbacher et al., 2004]. Additionally, major life stress (relocation to a new building) resulted both in increased HPA axis activation and self-injurious behavior [Davenport et al., 2008].

Although anxiety has been implicated as one factor in the maintenance of NSSI in humans [Klonsky et al., 2003; Ross & Heath, 2003], a relationship between anxiety and SIB has not been fully established in monkeys. Early life stress is known to be a risk factor in developing both anxiety and SIB disorders in monkeys [Lutz et al., 2003; Dettmer et al., 2012] but the two have not yet been connected. The only clear evidence that monkeys with SIB may have an anxiety disorder comes from pharmacological investigations with anxiogenic and anxiolytic compounds. Monkeys with SIB showed a mild increase in biting behavior with the anxiogenic drug FG7142 [Major et al., 2009] and a reduction in biting and wounding when treated with the anxiolytic drug, diazepam

[Tiefenbacher et al., 2005]. It should be noted, however, that only a subset of monkeys reacted to these drugs. Nearly half of the SIB monkeys showed no response to the anxiogenic drug FG7142 and half of the SIB monkeys treated with diazepam actually got worse. It is possible, as has been reported in the human population, that there may be different subtypes of SIB. These studies provide a framework to support the connection of SIB and anxiety that we further explore in our second experiment using the cage-side variant of the HIT.

The goal of this thesis is to gain further understanding of a commonly used anxiety assessment for rhesus macaques. We utilized the cage-side HIT in two experiments with the aims of: 1) testing two variations of procedure in two cage types and 2) more directly examining the hypothesis that monkeys with SIB might also show heightened anxiety compared to monkeys without this disorder.

CHAPTER 2

EXPERIMENT 1

2.1 Introduction

The first experiment had two objectives: 1) to determine whether the streamlined cage-side HIT elicits a significantly different response from monkeys than a more traditional HIT, and 2) to determine if cage structure influences how the monkeys react. The streamlined cage-side variant of the HIT is easily run in most laboratory facilities, although habituation to the intruder in the room, resulting from no alone phase between intruder phases, may lessen the reaction to the intruder's presence. If monkeys habituate to the intruder in the room during the cage-side HIT, then the stare phase of the traditional HIT where the intruder re-enters the room would elicit a stronger response than the stare phase of the cage-side HIT where the intruder remains in the room after the profile phase. We predicted that the more traditional variation of the HIT would provoke a stronger reaction to the stare of the intruder as manifested either by heightened aggressiveness or heightened anxiety (scratching, yawning, fear grimacing, freezing etc.). We ran both the cage-side HIT and a more traditional variation of the HIT using a within subjects randomized design to test this prediction. The cage-side HIT was previously run at three national primate centers as part of a study determining whether stress and anxiety were contributors to hair loss and abnormal behavior. We used the data from this study to compare the UMass monkeys to other laboratory-housed rhesus macaques in their reactions to the cage-side HIT.

In our analysis, we assessed if behaviors performed during the HIT could be predicted by cage structure. All monkeys were kept in their home-cage for both versions of the test. Approximately half of the monkeys were housed in floor-to-ceiling chain link pens with the remaining housed in Allentown cages, the standard housing environment for macaques at most major primate facilities. Since pens are larger, they allow the monkey to move farther away from the intruder during the test. This option of distance may create a scenario similar to tests that use multiple distances for intruder positions, with the farther distance being less threatening. If the monkeys perceive the intruder as more threatening when they are not able to move farther away from them, then monkeys in the smaller space of the Allentown cages would show a greater reaction (more threatening or freezing behavior) to the intruder. We also predicted that monkeys in pens would spend more of their time in the back half of the cage.

2.2 Methods

2.2.1 Subjects

The subjects were seventeen adult rhesus macaques housed at the University of Massachusetts Amherst. The subjects had an average age of 14 years with a range from 10 to 24 years. Four of the subjects were female. Seventy-five additional subjects from three other primate centers were added to this study for comparison. The distribution of subjects across the three facilities was: 31 subjects with an average age of 8.5 years from facility A (14 male), 24 subjects with an average age of 12 years from facility B (12 male), and 20 subjects with an average age of 11 years from facility C (1 male).

All subjects experienced the same daily enrichment routines, were fed Lab Diet Monkey Chow twice daily, had ad libitum access to water, were involved in a vigorous program of enrichment implemented daily, and were maintained in accordance with the National Research Council Guide for the Care and Use of Laboratory Monkeys.

2.2.2 Housing

All subjects were housed indoors in either Allentown cages (5 x 6 x 2 ft) or floor to ceiling pens (4 x 6 x 8 ft) in five separate rooms with a range of two to four monkeys per room. Table 2 breaks down the 17 subjects by housing condition. The subjects at the three other primate centers were housed in Allentown cages.

Table 2.1 Housing of subjects in experiment 1

	Pair housed	Grooming contact	Singly housed
Allentown	2	0	7 (75 from other facilities)
Pen	2	4	2

2.2.3 The Human Intruder Tests

All subjects received two versions of the human intruder test. During both tests pair-housed monkeys were separated and monkeys housed in Allentown cages were enclosed to one side of the cage. The monkeys were thoroughly habituated to this separation as part of daily husbandry procedures. All tests were video recorded. The intruder for each subject was the same female experimenter across both tests and was unfamiliar to the subject before the first test. At the beginning of both tests, a familiar experimenter entered the room to set up a camera, marked the spot of the floor for the intruder to stand, and separated the pair-housed subjects or restricted the individually housed monkeys to one half of their Allentown cages. Based on random assignment, nine monkeys received the cage-side HIT first and the traditional HIT three weeks later. The remaining eight monkeys received the traditional HIT first with the cage-side HIT run three weeks later.

The cage-side HIT consisted of a 10-minute baseline (camera-only) phase, of which the final two minutes was scored for behavioral analysis, followed by three 2-minute intruder phases. During the intruder phases, the intruder stood 0.6 meters from the subject's cage in three orientations in succession without leaving the room. The intruder initially stood orthogonal to the subject (profile), then turned to directly face the subject (stare), and then turned directly opposite to the subject (back). After the back phase the intruder left the room and the test was complete. A timer held by the intruder cued the start of each phase. The monkeys from other centers were only tested on the cage-side HIT.

The traditional HIT consisted of a 2-minute alone phase (alone 1), a profile phase run the same way as in the cage-side version, another 2-minute alone phase (alone 2), a stare phase run the same way as in the cage-side version, and then a final 2-minute alone phase (alone 3). Phases were timed with a timer outside of the room and signaled to the camera or intruder by a knock on the door. The intruder was instructed to have a completely neutral facial expression and to not look at any other monkeys in the room.

Table 2.2 Cage-side and traditional HIT phase order

Phase	**1**	**2**	**3**	**4**	**5**
Cage-side	10 minute Baseline	2 minute Profile	2 minute Stare	2 minute Back	____
Traditional	2 minute Alone	2 minute Profile	2 minute Alone	2 minute Stare	2 minute Alone

2.2.4 Behavioral Scoring

Videos were scored for duration using frame-by-frame analysis with the program Streaming MPEG. For each subject, behavior durations were rounded to the nearest second and summed to create totals for each phase. Inter-observer reliability was calculated to create an agreement score of >90% such that behavioral duration scores were within a range of 15-frames (equal to 0.5 seconds). The last two minutes of the baseline phase and the three 2-minute intruder phases of the cage-side HIT and all phases of the traditional HIT were scored for the duration of nine behaviors: back of cage, pace, freeze, lipsmack, teeth gnash, fear grimace, yawn, scratch, and threat/cage shake. Any other behaviors were noted but not scored for duration.

In addition to the categories of behavior described above, four broader categories were created for analysis. An anxiety category included the behaviors freeze, scratch, yawn, and fear grimace. A communication category included all facial expressions (i.e., lipsmack, teeth gnash, fear grimace, yawn, and threat/cage shake). Additionally, two global categories were created: range, to reflect how many unique different behaviors the monkeys expressed during each phase of the HIT; and change, which reflects the total number of times the monkeys switched from one behavior to another in each of the phases. All behaviors were scored for the additional three facilities, and the combined behavior categories (anxiety and communication) were created. However, range and change was only calculated from UMass data.

2.2.5 Data Analysis

<u>Phase Comparisons on Two HIT Procedures</u>: To analyze how the monkeys responded to the intruder in the two tests, an analysis of variance (ANOVA) was run for each HIT variant using phase as the within subjects variable. Initially, the data were tested for normality using the Shapiro-Wilks test, and where necessary, the scores were normalized using a log transformation. Scores for yawning, fear grimacing, and lipsmacking were infrequent and could not be analyzed statistically as a function of phase for both the

traditional and cage-side HIT analyses. For a similar reason, self-groom could only be analyzed during the cage-side HIT and threat/cage-shake during the traditional HIT.

Comparison of Response to Intruder by HIT procedure and Cage Type: To compare similar phases of the two test variants and assess the impact of cage style a mixed ANOVA was run. The profile and stare HIT phases of both cage-side and traditional variants were used as the within subject variable and cage style as the between subjects variable in this analysis. Fear grimace and lipsmack had scores too infrequent for statistical analysis. We used this method of analysis to analyze all other behaviors and behavioral categories.

Comparison of UMass monkeys with monkeys at other facilities: To determine if the UMass monkeys differed from monkeys at other facilities, a mixed ANOVA was run using HIT phase as the within subject variable and facility as a between subjects variable. The cage-side HIT was used for this comparison. In this analysis, the focus was on the interaction of facility with phase and not on the main effects of phase. Fear grimace and lipsmack could not be analyzed when comparing across facilities because of the sparse number of scores.

2.3 Results

The first analysis focused on phase differences within each HIT procedure (see table 4). In the cage-side HIT, there were no significant effects of phase. In the traditional HIT, only one significant effect was detected. Monkeys paced significantly more during the alone phases ($F = 3.530$, $p = 0.011$) with an average of 4.765 seconds during the intruder phases and 18.235 seconds during the alone phases (see figure 1). It should be noted that only six out of the seventeen monkeys paced during the traditional HIT.

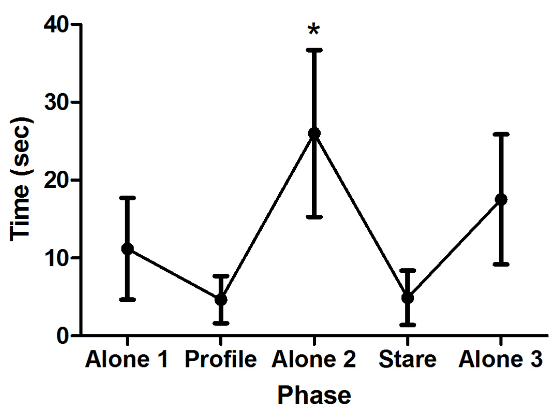
Table 2.3 Phase effects of cage-side and traditional HIT

Behavior or Category	Traditional HIT	Cage-side HIT
	P-values for Main Effects of Phase	
Back of cage	ns	ns
Pace	0.011	ns
Freeze	ns	ns
Lipsmack	x	x
Teeth gnash	ns	ns
Fear grimace	x	x
Yawn	x	x
Scratch	ns	ns
Threat / cage shake	ns	x
Self groom	x	ns
Anxiety	ns	ns
Communication	ns	ns
Change	ns	ns
Range	ns	ns

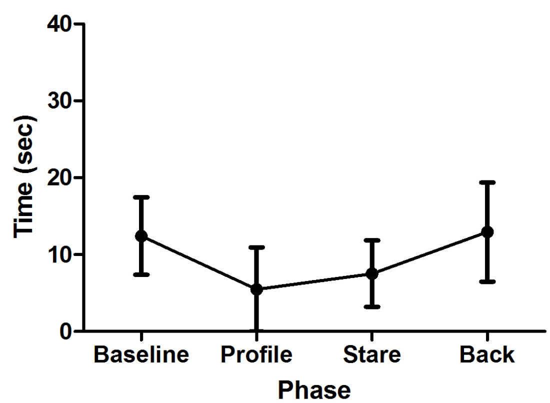
ns (non significant) p-value > 0.05
x means too many scores were zeros, preventing statistical analysis

Figure 2.1 A main effect of phase was seen for pacing during the traditional HIT, this effect was not seen in the cage side HIT

a.



b.



a) Pacing during the 5 phases of the traditional HIT showed significant main effect of phase. The significant difference was seen when comparing the intruder phases to the second alone phase ($F = 3.530$, $p = 0.011$).
b) Pacing during the cage-side HIT showed no significant phase effects ($F = 2.588$, $p = 0.064$)

In the second analysis we compared the intruder phases (profile and stare) of the traditional HIT and cage-side HIT as a function of cage style. We predicted that monkeys would show a stronger reaction to the intruder phases of the traditional HIT as compared to the cage-side HIT. However, monkeys showed no significant difference in behavior between the two variants of the HIT (see table 5). Contrary to predictions, cage structure (pen vs. Allentown Cage) did not influence how monkeys responded to the HIT (see table 5).

Table 2.4 Effects of cage style and phase between HIT variants

Behavior / Category	**Main Effect of Phase**	**Main effect of cage style**	**Phase x cage style interaction**
Back of cage	ns	ns	ns
Pace	ns	ns	ns
Freeze	ns	ns	ns
Lipsmack	x	x	x
Teeth gnash	ns	ns	ns
Fear grimace	x	x	x
Yawn	ns	ns	ns
Scratch	ns	ns	ns
Threat / cage shake	ns	ns	ns
Self groom	ns	ns	ns
Anxiety	ns	ns	ns
Communication	ns	ns	ns
Change	ns	ns	ns
Range	ns	ns	ns

ns (non significant) p-value > 0.05
x means too many scores were zeros, preventing statistical analysis

In the third analysis, we examined whether the UMass monkeys were different than rhesus macaques at three other facilities (see table 6). In this analysis, we used only the cage-side HIT data. We were primarily interested in the interaction of facility with phase. There were several main effects of facility (noted below in Table 2.5), but in every case, the interaction was also significant (see Appendix for graphs), and thus we discuss only the interactions.

Table 2.5 Interaction effects of facility and phase (see last column)

Behavior / Category	Main Effect of Facility	Main effect of Phase	Facility x phase
Back of cage	ns	< 0.001	< 0.001
Pace	ns	< 0.001	ns
Freeze	ns	< 0.001	0.006
Lipsmack	0.001	< 0.001	< 0.001
Teeth gnash	ns	< 0.001	0.003
Fear grimace	x	x	x
Yawn	ns	< 0.001	0.011
Scratch	< 0.001	ns	0.004
Threat / cage shake	0.022	< 0.001	0.001
Anxiety	0.020	0.001	0.002
Communication	ns	< 0.001	< 0.001

Monkeys at UMass showed a significantly different pattern of reactivity compared to macaques at three other facilities. When examining the amount of time they spent in the back of the cage there was a significant phase by facility interaction ($F = 3.763$, $p < 0.001$). While at the three comparison facilities, monkeys went to the back of the cage when the intruder entered the room, monkeys at UMass approached the intruder and spent time near the intruder when she was in the room (see figure 2). There was also a significant interaction between facility and phase in the amount of time monkeys spent freezing ($F = 2.637$, $p = 0.006$). Monkeys at UMass showed relatively low freezing behavior across the test, similar facilities B and C (see figure 3).

Figure 2.2 A significant phase by facility interaction was seen in the amount of time monkeys spent in the back of the cage

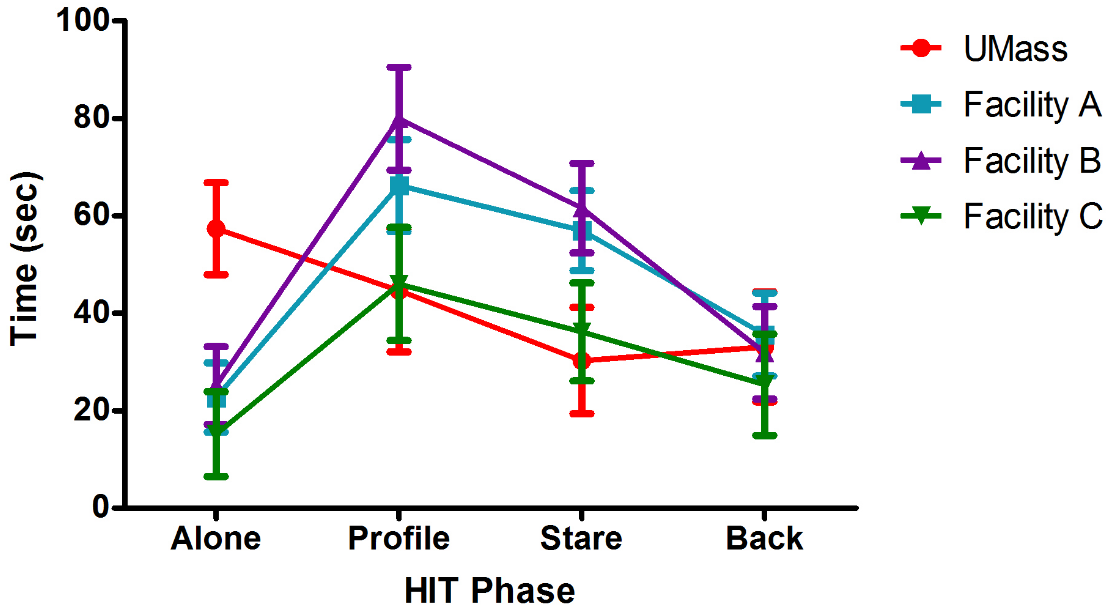
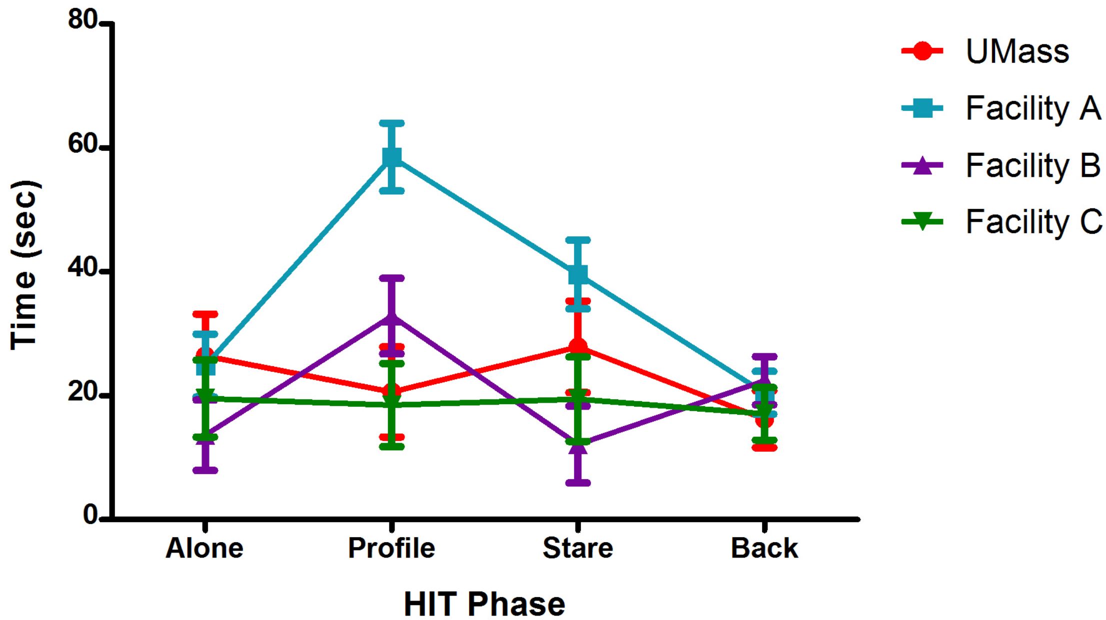


Figure 2.3 A significant phase by facility interaction was seen in the amount of time monkeys spent freezing



There was a facility by phase interaction for both lipsmacking and teeth gnashing behaviors (Lipsmack: $F = 5.703$, $p < 0.001$; Teeth gnash: $F = 2.870$, $p = 0.003$). Lipsmacking occurred rarely out of the stare phase. UMass monkeys showed low scores across the test, similar to facilities A and C (see figure 4). Teeth gnashing behavior at the UMass facility was more consistent across phases than at the other facilities (see figure 5).

Figure 2.4 A significant phase by facility interaction was seen in the amount of time monkeys spent lipsmacking

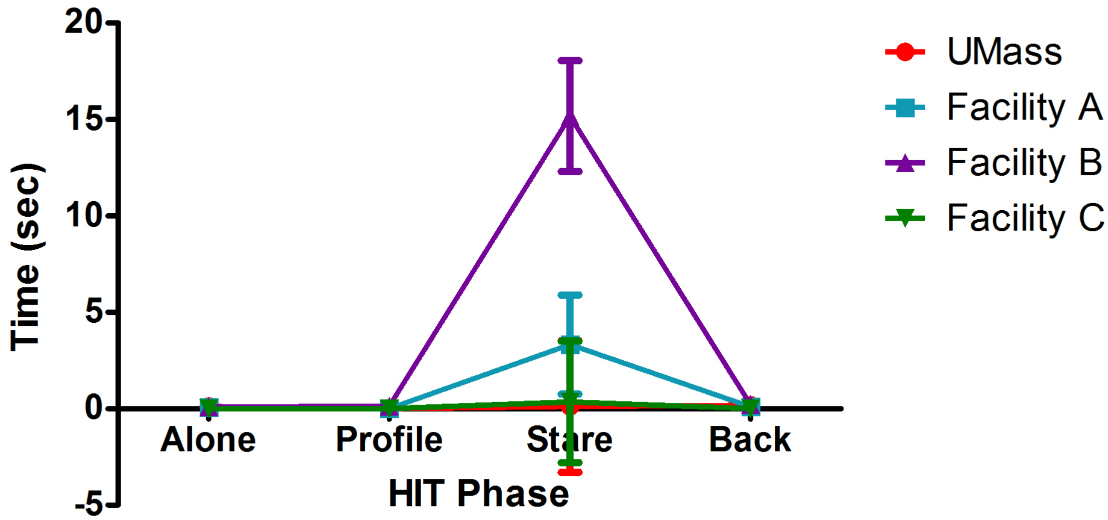
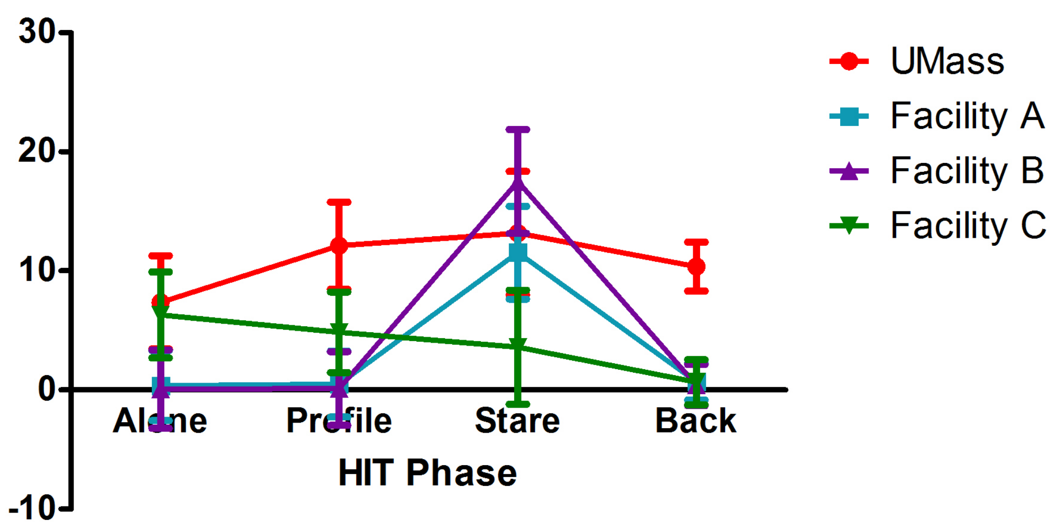
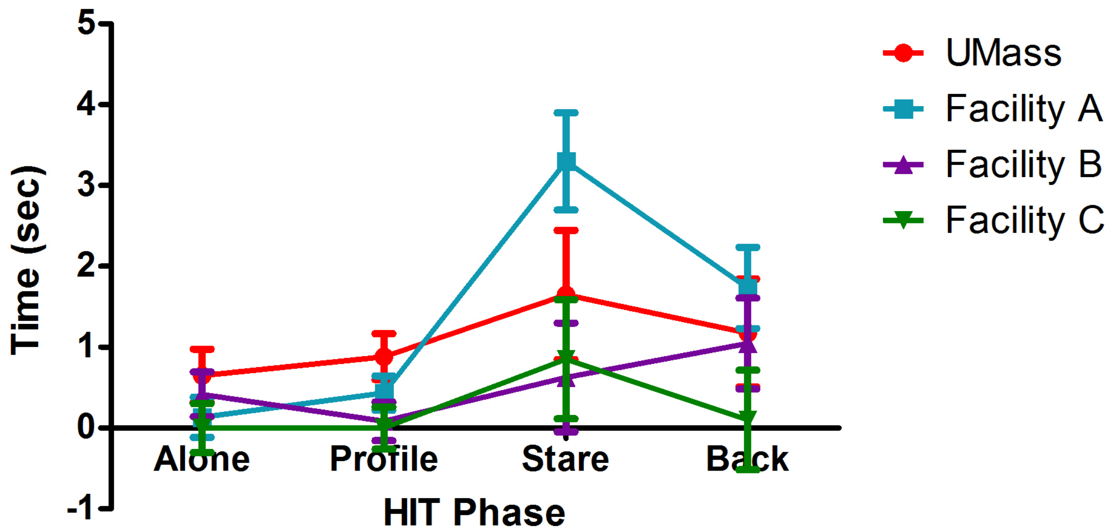


Figure 2.5 A significant phase by facility interaction was seen in the amount of time monkeys spent teeth gnashing



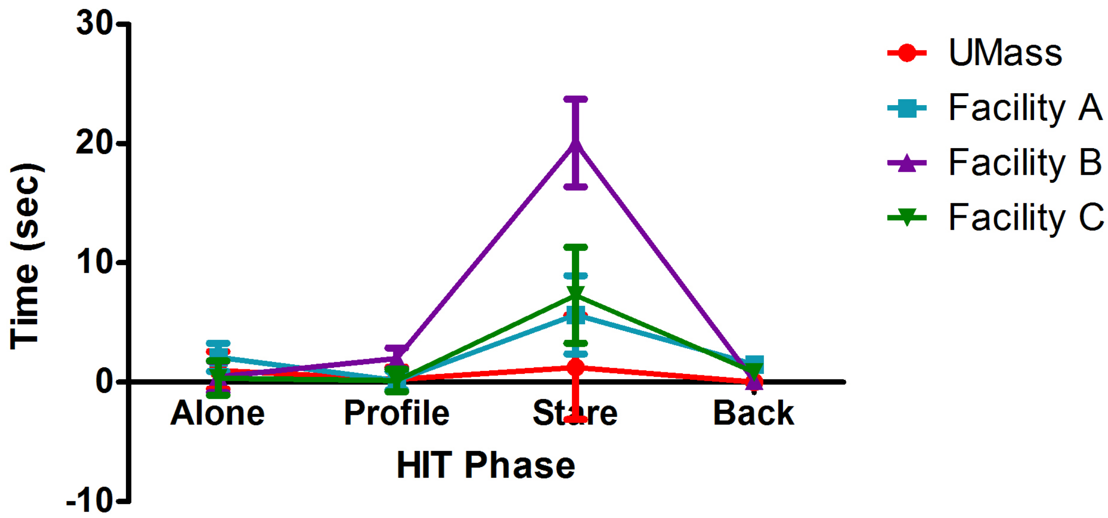
A significant facility by phase interaction was found for yawning behavior (F = 2.438, p = 0.011). Time spent yawning increases in the stare phase for facility A, while at UMass, facility B, and facility C yawning durations stay relatively low throughout the test (see figure 6).

Figure 2.6 A significant phase by facility interaction was seen in the amount of time monkeys spent yawning



UMass monkeys showed very little aggressive behavior during the HIT with a total average across all four phases of <1 second. A significant phase by facility interaction is seen for threat/cage shake behavior (F = 4.042, p < 0.001). The majority of threat/cage shake behavior occurred during the stare phase of the test, although, only facility B shows a significant increase (see figure 7).

Figure 2.7 A significant phase by facility interaction was seen in the amount of time monkeys spent threatening or shaking the cage

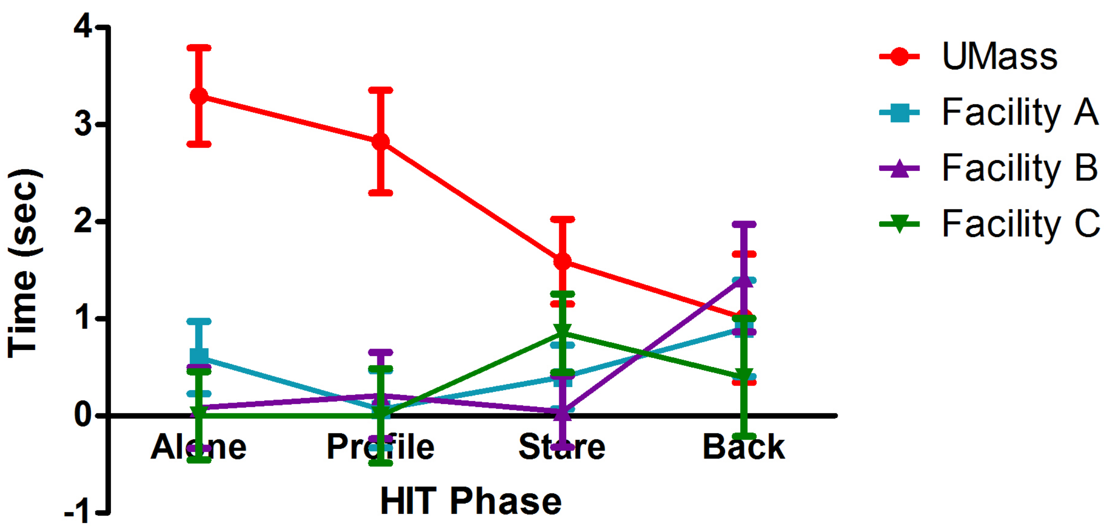


The communications category of behavior included teeth gnashing, lip smacking, yawning, threat/cage shake, and fear grimacing. This category of behavior showed a significant facility by phase interaction ($F = 4.531$, $p < 0.001$). Facility B shows a significant increase in communication behaviors during the stare phase, following the trend of the individual behaviors lipsmack, teeth gnash, and threat / cage shake. UMass, as with the individual behaviors, showed relatively low scores similar to facilities A and C (see figure 8).

Figure 2.8 A significant phase by facility interaction was seen in the amount of time monkeys spent performing communication behaviors

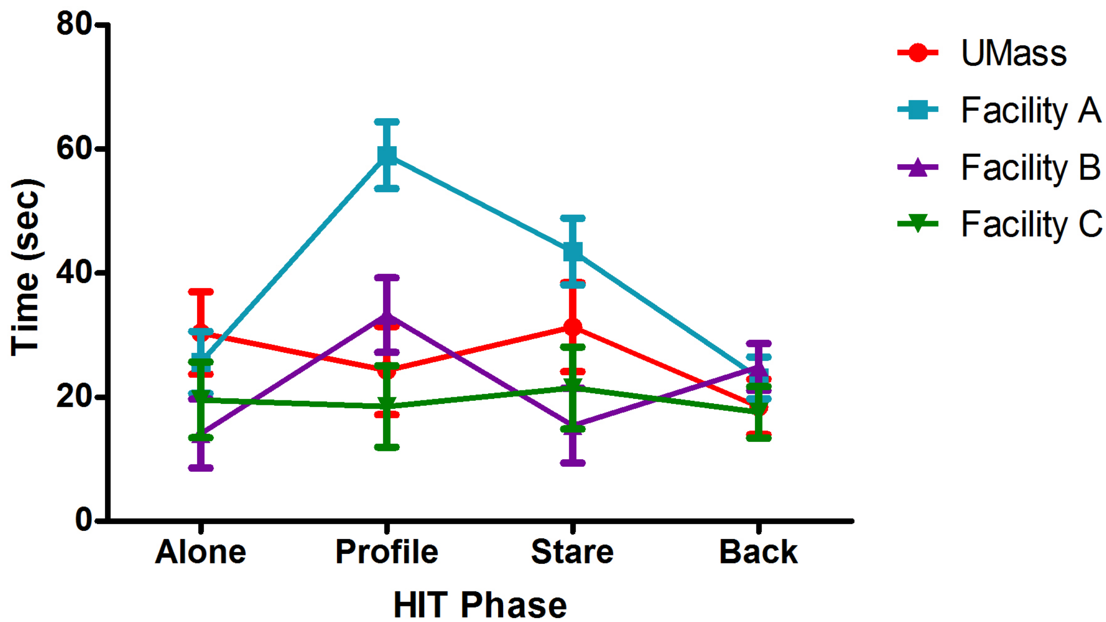
Monkeys at UMass showed a significantly different pattern of scratching behavior throughout the HIT when compared to the other facilities (F = 2.746, p = 0.004). While at the additional three facilities scratching occurred for an average of <1 second per phase, UMass monkeys scratched an average of 2.178 seconds per phase, with more time spent scratching before the intruder entered the room (see figure 9).

Figure 2.9 A significant phase by facility interaction was seen in the amount of time monkeys spent scratching



Facility mediated the relationship between anxiety behavior and phase ($F = 3.076$, $p < 0.002$). The UMass monkeys showed a similar amount of anxious behavior during the test as facilities B and C. Facility A had a significant increase in anxiety behaviors when the intruder entered the room (see figure 10).

Figure 2.10 A significant phase by facility interaction was seen in the amount of time monkeys spent displaying anxiety behaviors



2.4 Discussion

Contrary to predictions, UMass monkeys did not respond differently across phase to the two HIT procedures. Neither variation of the HIT elicited the expected responses: aggression toward the intruder, freezing, or anxious behaviors seen in previous studies [Kalin et al., 1998; Capitanio, 1999; Corcoran et. al., 2012]. In fact, there was very little reaction to the intruder regardless of HIT type. Pacing was the only behavior that showed a significant pattern in response to the intruder. This effect was seen in the traditional variation of the HIT, but not the cage-side variation. Pacing, often considered an abnormal behavior in rhesus macaques, is the most common stereotypy seen in the species in captivity [Lutz et al., 2003]. In this case, subjects suppressed their pacing when the intruder was in the room. One possible reason for the pattern of pacing during the HIT is that attending to the intruder and pacing may be mutually exclusive. Contrary to predictions, a direct comparison of the two procedures during the two intruder phases (Profile and Stare) yielded no significant effects. In this analysis, cage size also failed to yield an effect.

Because of these findings, we then compared the UMass monkeys with monkeys from different facilities. This comparison yielded many significant interactions. The pattern of time the UMass monkeys spent in the back of the cage while the intruder was in the room suggests that they had a different reaction to the intruder. When compared to monkeys at other facilities, the monkeys at UMass showed a unique pattern. Retreating to the back of the cage during intruder phases is a commonly reported response to the HIT [Mason et al., 2006] and was observed in monkeys at the three other primate centers. In contrast, monkeys from the UMass facility came to the front of the cage during the cage-side HIT when the intruder entered the room. Although threatening and aggressive behavior, such as cage shaking, often occurred in the front of the cage directed at the intruder, this is not an explanation for the UMass monkeys spending less time in the back of the cage. Compared to the other three facilities monkeys from the UMass facility showed the lowest amount of aggressive behaviors.

Another explanation for these results may be a habituation to human experimenters and generalization of this habituation to new experimenters. Monkeys at the UMass facility are exposed to many different people on a daily basis. Each semester (about every six months) the monkeys at the UMass facility are exposed to new students acquiring skills in behavioral observation. Habituation occurs when over time a novel stimulus becomes familiar and no longer elicits a strong response [Clay et al., 2009]. Although the individual who acted as the human intruder during the test was truly novel to the monkeys, the HIT may not have generated the stressful or threatening experience for these monkeys as it may have done at other facilities where human contact is considerably lower.

It is possible that habituation to humans may also occur as a result of early life experience in some of the UMass subjects. About half of the UMass monkeys experienced nursery rearing and increased human contact in their first six months of life. However, a subsequent analysis comparing the behavior of nursery-reared vs. mother-reared monkeys on the HIT yielded only one significant difference out of the twenty-four ANOVAs run. During the cage-side HIT, nursery-reared monkeys spent more time in the front of the cage than mother-reared monkeys regardless of phase. However, this effect was not seen in the traditional HIT and therefore a comparison of early rearing history failed to reveal substantial differences in behavior. This suggests that the effect of continual habituation to novel experimenters exceeds any effect that rearing experience may have.

A non-reactive but attentive response to the intruder shows not only possible habituation to the intruder, but may demonstrate evidence of desensitization to the threat of a novel human. Desensitization occurs when a negative stimulus is paired with a positive stimulus over time resulting in the reduction of stress related behaviors [Clay et al., 2009]. The monkeys from the UMass facility participate in many cognitive tasks involving food treats. New students assist in administering the enrichment program as well as the cognitive tasks. Desensitization to a human intruder may have occurred in monkeys who were extremely fearful of new experimenters when they first came to this facility as a result of this exposure. A conditioned response, as a result of the rewarding cognitive tasks and enrichment programs, may now be seen as being attentive and coming forward in the cage when humans are in front of their cage. The monkeys from the UMass facility may have been anticipating a food reward when the intruder entered the room. Desensitization and habituation may preclude the unconditioned response the HIT is designed to provoke [Coleman & Pierre, 2014].

Although reactivity to the intruder was low in UMass monkeys, scratching behavior occurred significantly more during the baseline and profile phases than at other facilities. The camera was set up in the room with the monkey after which a 10 minute baseline ensued. The UMass monkeys are exposed to cognitive testing which involves setting up a camera and the experimenter entering the room with apparatus in about 2 minutes. With routine exposure to the camera during cognitive tasks, UMass monkeys may have anticipated a cognitive task to be starting when the camera was set up. Scratching during the last two minutes of baseline may be a result of their expectation being violated. Studies with human infants and one study with dogs demonstrate an increased attention to unexpected outcomes utilizing violation-of-expectation paradigms [Erdohegyi et al., 2009; Tee & Dissanayake, 2011]. When the intruder entered the room the monkeys may still have been expecting a cognitive task causing them to come forward. This may be another explanation for the UMass monkeys' attention to the intruder as well.

The amount of early human exposure may also play a role in how the monkeys react to the test. However, when looking at reaction to the intruder as a function of rearing condition (nursery or mother reared) UMass monkeys did not show a great difference in how the monkeys reacted to the intruder. The only significant difference was that nursery reared monkeys spent significantly more time in the front of the cage throughout the test.

CHAPTER 3

EXPERIMENT 2

3.1 Introduction

The second aim of this thesis was to determine if monkeys that have a history of SIB are more anxious as measured by the cage-side HIT. If anxiety were a component of the SIB pathology, than monkeys with a record of SIB would be more anxious than controls in response to the mild stress of the human intruder. We predicted that monkeys with SIB would display more anxious behaviors including fear grimacing, freezing, scratching and yawning than controls throughout the test, display more aggressive behavior when threatened by the intruder, and would spend more time in the back of the cage avoiding the intruder during the two intruder phases. Our objective was to gain a better understanding of any relationship between SIB and monkey emotional disposition using the HIT.

3.2 Methods

3.2.1 Subjects

Subjects were rhesus macaques from three national primate centers: Washington National Primate Research Center, New England Primate Research Center, and the Laboratory of Comparative Ethology in NIH Poolesville, Maryland. Forty-one subjects that exhibited SIB, either biting or wounding, were identified using veterinarian or technician records. Thirty-eight control subjects were chosen to match the facility, age, and gender of the SIB subjects. Two control subjects had to be dropped because of poor quality recordings. Thirty-seven of the remaining 77 subjects were male, and all subjects were between the ages of 4 and 24 with a mean age of 9.9 years. All 77 subjects were housed indoors, experienced similar enrichment routines, were fed chow twice daily, had water ad libitum, and were kept in accordance with the National Research Council Guide for the Care and Use of Laboratory Monkeys.

Table 3.1 Demographic information on subjects in experiment 2

Facility	SIB		Control	
	Male	Female	Male	Female
Washington	10	20	10	19
New England	9	0	5	0
Maryland	2	0	2	0
All Facilities	21	20	17	19
Total	41		36	

3.2.2 The Human Intruder Test

All subjects received the cage-side HIT. The test was run the same way at all facilities and was video recorded. The intruder was a female experimenter unfamiliar to the subject. All pair-housed subjects were separated from each other for the duration of the test. At the beginning of the test, a camera was placed in the room. The test consisted of a 10-minute baseline (camera only) phase followed by three 2-minute intruder phases. During the intruder phases the intruder stood approximately 0.6 meters from the subject's cage in three orientations without leaving the room. The intruder initially stood orthogonal to the subject (profile), then turned to directly face the subject (stare), and then turned directly opposite to the subject (back). After the back phase the intruder left the room and the test was complete. A timer held by the intruder cued the start of each phase.

3.2.3 Behavioral Scoring

Videos from all three facilities were sent to the University of Massachusetts Amherst to be scored centrally. Videos were scored using frame-by-frame analysis using Streaming MPEG. All scorers were blind to the facility and SIB status of the subjects. All behaviors were scored for duration. Inter-observer reliability was calculated as > 90% agreement scores such that behavioral duration scores were within a range of 15-frames (equal to 0.5 seconds). The last two minutes of the baseline phase and the three 2-minute intruder phases were scored for the duration of fifteen behaviors: back of cage, pace, freeze, lipsmack, teeth gnash, fear grimace, yawn, scratch, threat/cage shake, self-groom, self-bite, eye poke, floating limb, self-mouth, and self-sex.

Table 3.2 Behavioral scoring ethogram

Behavior	**Definition**
Back of cage	At least three limbs occupy the back half of the cage (farthest from intruder) for more than 45 frames.
Pace	Repeated locomotor movement 3 or more times.
Freeze	No movement for 60 frames or more
Lipsmack	When mouth is puckered and moving quickly up and down to produce a smacking sound. Often paired with eyebrows and ears back.
Teeth Gnash	A chewing motion of the mouth with no food or objects involved.
Fear Grimace	Grin-like facial expression with lips drawn back showing clenched teeth. Can be paired with flapping of ears and stiff body posture.
Yawn	A slow opening of the mouth to an extremely wide position often exposing the teeth.
Scratch	A vigorous stroking of the body with nails.
Cage-shake/threat	Open mouth stare with teeth partially exposed, eyebrows lifted, ears flattened or flapping, rigid body posture, lunging toward the front of the cage, shaking cage vigorously, slapping cage, bouncing off walls of cage
Self groom	Any picking, scraping, spreading, licking, or mouth picking of hair or nails.
Self-bite	Vigorous biting of any part of the body.
Eye poke	Placing hand or fingers near the eyes.
Limb float	Holding a limb in the air.
Self sex	Any oral or tactile manipulation of the genitals.
Self mouth	Any sucking or mouthing of the body.
Other	Crooktail, tac oral, forage, present, drink, eat, or any other undefined behavior

3.2.4 Data Analysis

The following behaviors were analyzed: back of cage, pace, anxiety, threat/cage shake, self-directed stereotypy, and self-groom. Additionally, two global categories were created: range which reflected the number of unique behaviors expressed during each phase of the HIT, and change which reflected the total frequency of all behavioral events regardless of whether they were repeated, during each phase.

For each subject, behavior durations were rounded to the nearest second and summed to create totals for each phase. Mixed analysis of variances (ANOVAs) were run using HIT phase as the within subject variable and SIB status and sex as the between subjects variables.

3.3 Results

Seven of the subjects identified as having SIB showed mild biting behavior during the HIT, but no wounding occurred. There was no common phase in which the biting occurred. In fact, for two subjects, biting occurred once during the baseline and for two it occurred once or twice during the back phase. Two subjects bit once during the stare phase. The remaining subject bit several times during the phases in which the intruder was in the room.

As expected, threatening behaviors varied across phase ($F(3,225) = 12.994$, $p < 0.001$) with most of the threatening behaviors occurring during the stare phase. However, contrary to predictions, monkeys with SIB showed significantly lower aggressive behavior during the stare phase ($F(3,225) = 3.340$, $p < 0.05$) and also showed a trend for lower aggression across the entire test ($F(1,75) = 3.808$, $p = 0.055$) (see figure 11). Contrary to predictions, SIB monkeys spent less time in the back of the cage overall than control subjects ($F(1,75) = 4.195$, $p < 0.05$). Time spent in the back of the cage was significantly increased with the intruder's presence in the room ($F(3,225) = 12.175$, $p < 0.001$), but SIB subjects spent significantly less time in the back of the cage when the intruder entered the room compared to controls ($F(3,225) = 3.255$, $p < 0.05$) (see figure 12). The HIT also elicited a phase dependent anxiety response ($F(3,225) = 5.188$, $p < 0.01$) as expected. However, contrary to predictions, monkeys with SIB did not differ from controls in their anxious behavior (see figure 13).

SIB status had no effect on pacing or freezing behavior. Across all subjects pacing decreased during the intruder phases of the test by an average of six seconds compared to the baseline ($F(3,225) = 3.364$, $p < 0.05$) and freezing increased during the intruder phases, with the most freezing occurring during the stare phase ($F(3,225) = 4.677$, $p < 0.01$). We predicted that SIB subjects would spend more time scratching throughout the entire test than controls. However, this difference was marginal ($F(1,75) = 3.672$, $p = 0.059$), and the difference between the two groups was less than one second in duration. No significant differences as a function of SIB status or phase were detected for yawning, self-grooming, or self-directed stereotypies.

Because monkeys with SIB behaved unexpectedly by spending *less time* displaying some of the target behaviors than controls, we explored two features of the overall behavioral repertoire: 1) the number of unique behaviors expressed during the HIT (range), and 2) the total number of behavior scores (change). The range of behaviors varied by HIT phase with the highest number of unique behaviors performed during the stare phase (average = 2.909) and the lowest number of unique behaviors performed during the profile phase (average = 2.052) ($F(3,225) = 6.299$, $p < 0.001$). Additionally, males showed a larger range of unique behaviors throughout the test ($F(1,73) = 7.604$, $p < 0.01$) than females. However, the range of unique behaviors was unaffected by SIB status.

As was the case for range, the behavioral change score varied as a function of the HIT phase and was highest during the stare phase ($F(3,225) = 17.859$, $p < 0.001$). Unlike the range, however, monkeys with SIB showed a significantly lower change score across all the HIT phases than controls ($F(1,75) = 4.662$, $p < 0.05$). This difference was most pronounced during the stare phase ($F(3,225) = 3.475$, $p < 0.05$) (see figure 14).

Figure 3.1 Mean time in seconds spent threatening the intruder in SIB (dotted line) and control (solid line) monkeys during the four phases of the HIT.

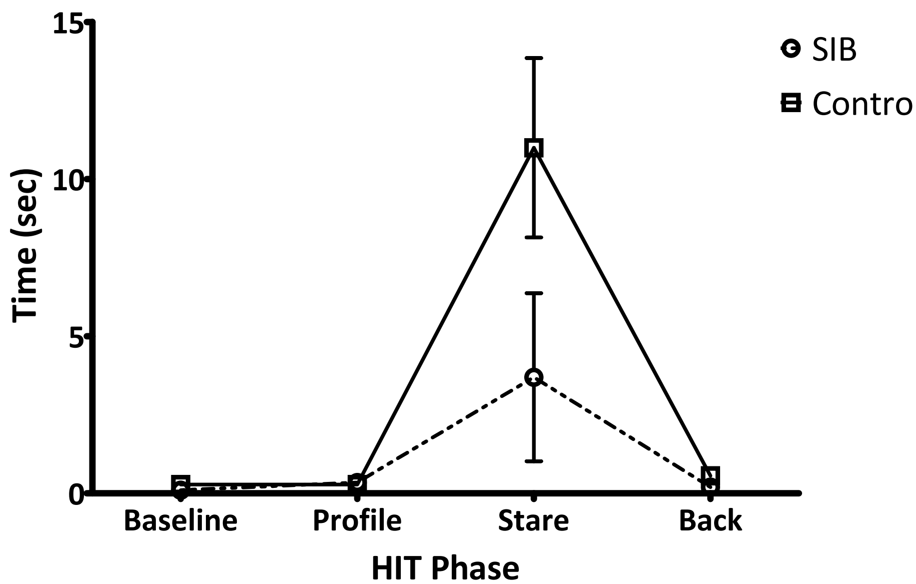


Figure 3.2 Average time in seconds spent in the back of the cage in SIB (dotted line) and control (solid line) monkeys during the four phases of the HIT.

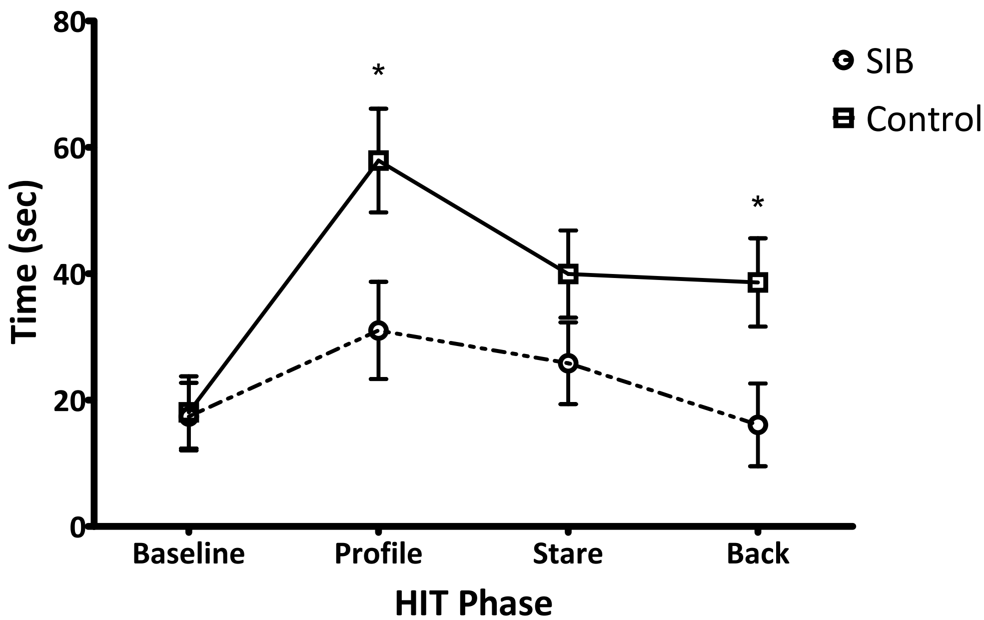


Figure 3.3 Average time in seconds spent exhibiting anxiety behaviors in SIB (dotted line) and control (solid line) monkeys during the four phases of the HIT.

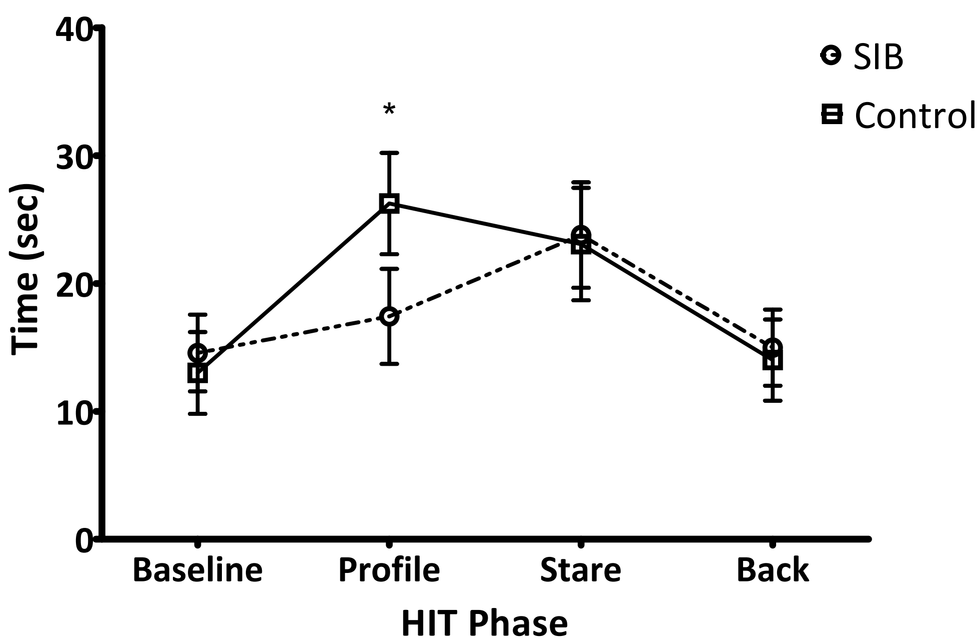
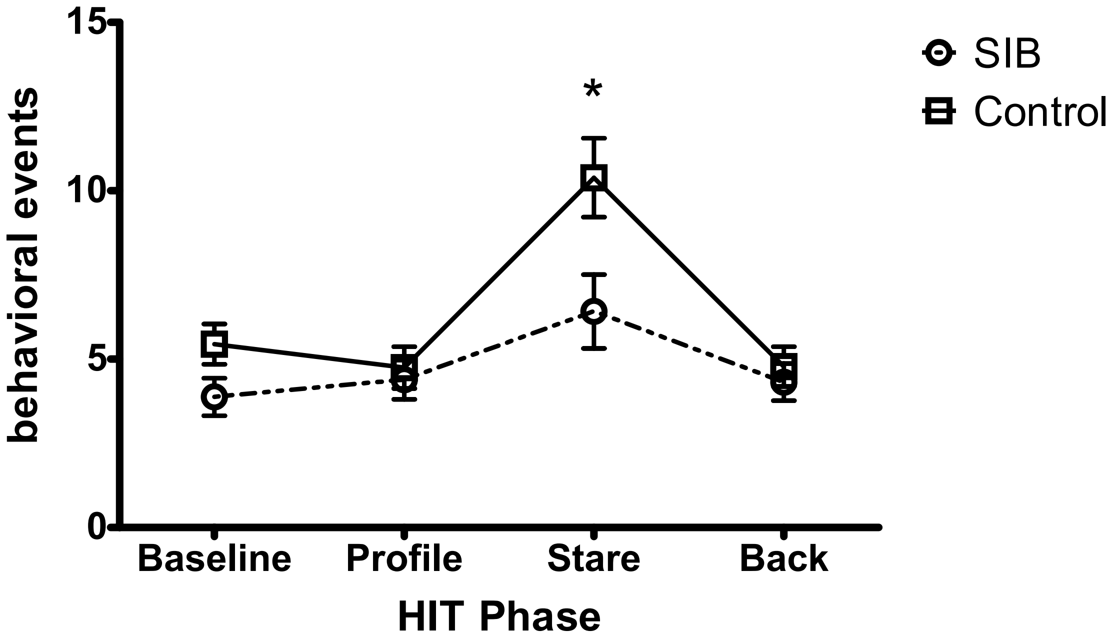


Figure 3.4 The total number of behavioral events displayed by SIB (dotted line) and control (solid line) monkeys during the four phases of the HIT.



3.4 Discussion

Our results add to the enigma of the SIB-anxiety relationship in monkeys. Previous pharmacological research on SIB has pointed toward a possible link. However, we found little evidence to suggest that monkeys with SIB are more anxious than controls, as measured by their reactions to the HIT. In fact, the data revealed that monkeys with SIB were significantly less reactive on this test than controls. Although the HIT elicited the expected defensive responses by control subjects particularly during the stare phase, we found that monkeys with a record of SIB were much less defensive. Additionally, although controls retreated to the back of the cage when the human intruder entered the room for the profile phase, this response was much lower in monkeys with a record of SIB. Overall, SIB subjects showed the same range of species-typical behavior as controls but changed from one behavior to another less frequently than control subjects. General levels of biting behavior for these subjects was not known, but biting was infrequent during the test. In general, the findings suggest a blunting of affect to the presence of the intruder. These findings are consistent with the blunting of the stress response system detected in another group of monkeys with SIB [Tiefenbacher et al., 2004], and are inconsistent with the view that monkeys with SIB are more anxious.

One explanation is that SIB monkeys may be habituated to the presence of observers. At large primate facilities, veterinary and care staffs do not spend significant time observing monkeys on a weekly basis. For most monkeys the HIT is a truly novel situation. Monkeys with SIB often need special treatment or observation by humans in order to monitor their pathology. With frequent observers, SIB monkeys may not perceive the human intruder to be novel enough to feel threatened and respond.

An alternative explanation is that these monkeys may show some form of depression. As with anxiety, depressive symptoms in humans are quite heterogeneous. However, blunted affect is one of several characteristic of major depressive disorder [Loas et al., 1994; Foti et al., 2010]. At least two studies report a relationship between depressive symptoms and NSSI in teenagers [Nock et al., 2006; Baetens et al. 2015]. Additionally, Asarnow and colleagues reported a relationship between NSSI and treatment-resistant depression [Asarnow et al., 2011]. At present, we cannot determine whether the SIB monkeys in this study were depressed.

Although behavioral manifestations of depression in non-human primates can be more challenging to discern than in humans, depressive symptomatology has been explored in monkeys. A depressive posture identified in macaques includes the head below the shoulders with the back slumped, similar to when sleeping, but with the eyes open [Shively & Willard, 2012]. Observing the amount of time monkeys spend in this collapsed posture could help further illuminate if monkeys with SIB are more likely to have a depressive disorder. Anhedonia is a symptom of depression in humans that can also be used to identify depression in macaques. Anhedonia, as measured by a decreased consumption of sucrose, is correlated with disrupted dopamine release patterns in the striatum of macaques [Felger et al., 2013]. This evidence supports anhedonia as another valid depressive symptom to explore in macaques with SIB.

Our results suggest that anxiety as measured by the HIT may not be part of the macaque SIB pathology; instead, there may be a connection with depression. However, in humans 50% of depressed patients also have anxiety [Hirschfeld, 2001]. If both depression and anxiety can be comorbid in macaques, it is difficult to know whether a possible depressive phenotype may have masked an anxious response on the HIT.

A greater understanding of the SIB pathology in macaques would have value for the use of macaques as a translational model, as well as having implications for improving the welfare of laboratory macaques. Monkeys with SIB are a challenge to care for, and treatment outcomes are quite variable. Further exploration into a possible depressive-like behavioral phenotype in monkeys with SIB should be explored. The presentation of huddled depressive-like posture and anhedonia in monkeys with SIB would support the hypothesis that SIB in macaques is associated with depression as it is in some adolescent humans.

CHAPTER 4

CONCLUSION

The results of these two experiments provide evidence that the HIT may not be a valid anxiety assessment for all laboratory-housed rhesus macaques. Although rhesus macaques are a neophobic species, and typically find novel humans to be a mild threat, their everyday environment dictates what is truly novel to them. The results of these two experiments indicate that captive rhesus monkeys that are exposed to novel humans regularly may not find a new human in front of their cage to be as much of a threat. Monkeys with SIB, who may often see new veterinary staff, and monkeys that frequently are involved in human administered cognitive testing, may have more routine exposure to new people. Future studies should explore more tests of reactivity and anxiety in these groups of monkeys to confirm the idea that habituation plays a role in a low reactivity level in response to the HIT.

Other provoked response test paradigms have been designed to utilize novel environments, objects, and social situations to assess anxious behavior. Novel environment tests, such as the open field test used in rodents, examines the behavior of an animal in a new environment [Coleman & Pierre, 2014]. Novel object tests involve exposing subjects to a range of neutral to threatening objects and assessing their reaction to the object and their latency to approach the object [Fairbanks & Matthew, 2011]. Objects with eyes, such as a Mr. Potato Head doll, may have a novel social aspect to them as well. Although the HIT is the most commonly utilized novel social paradigm, another novel social paradigm called the intruder challenge test utilizes an unfamiliar monkey to the subject in a similar way the HIT utilizes a novel experimenter [Fairbanks, 2001]. A mirror could also be utilized to create a unique novel social paradigm, as monkeys do not recognize mirror reflections as themselves but react as if the reflection was a novel monkey [Gallup & Suarez, 1991]. Our HIT results may still be an accurate anxiety assessment for these individuals if another provoked response test demonstrates the monkeys are low reactors when the situation is truly novel.

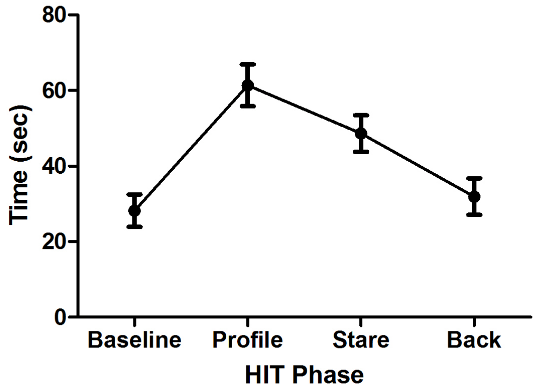
A noninvasive tool for assessing anxious individuals may be an important component of improving welfare in captive primate facilities. Anxiety can negatively influence both behavior and physiology leading to destructive behaviors or health problems. Identification of anxious individuals is the first step to decreasing anxiety and addressing these issues.

APPENDIX A

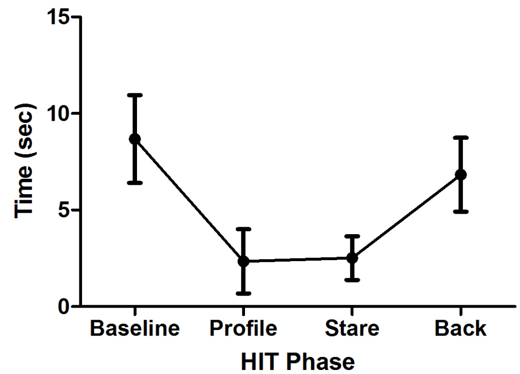
FACILITY EFFECTS

Main effects of phase across all facilities

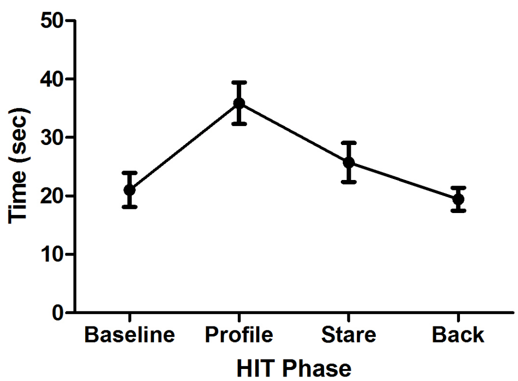
a. Back of cage



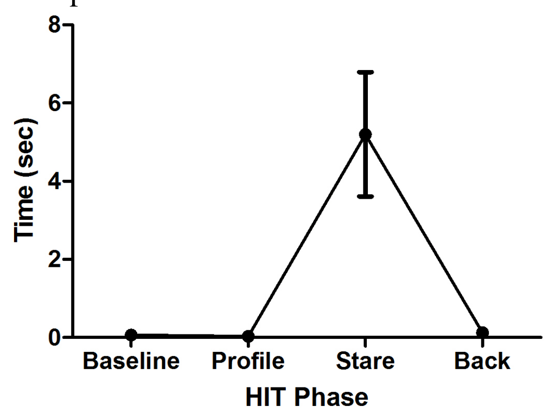
b. Pace



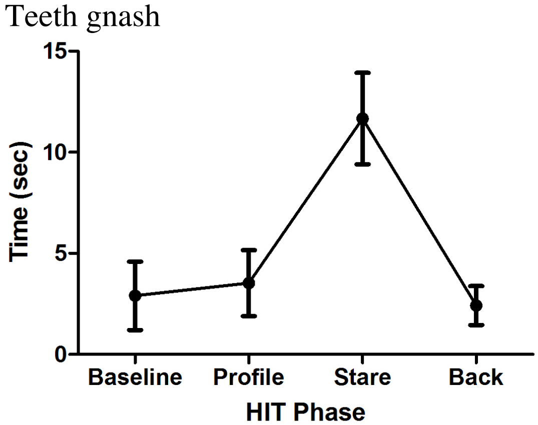
c. Freeze



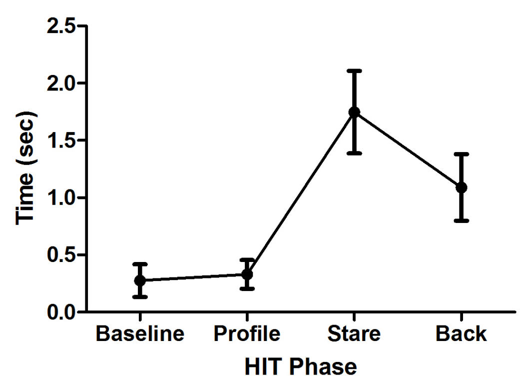
d. Lipsmack



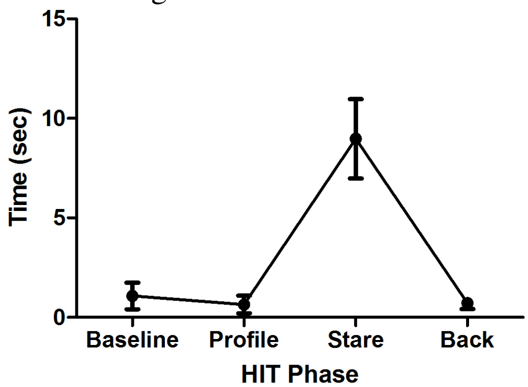
e. Teeth gnash



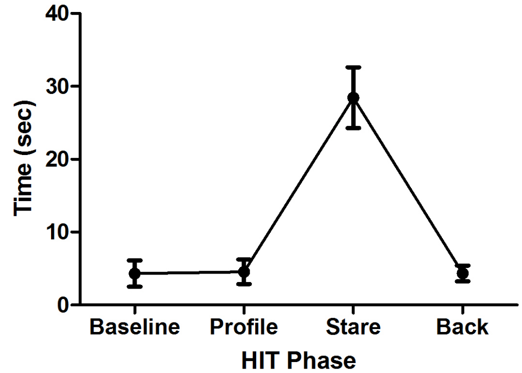
f. Yawn



g. Threat / cage shake



h. Communication



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