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The Effects of Predictability in Daily Husbandry Routines on Captive Rhesus Macaques (*Macaca mulatta*)

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Abstract

Rhesus macaques (*Macaca mulatta*) housed indoors experience many routine husbandry activities on a daily basis. The anticipation of these events can lead to stress, regardless of whether the events themselves are positive or aversive in nature. The specific goal of this study was to identify whether increasing the predictability of husbandry events could decrease stress and anxiety in captive rhesus macaques. This study was conducted on 39 single-housed subjects in four indoor rooms at the Oregon National Primate Research Center. Temporal and signaled predictability were added to four daily husbandry events: morning and afternoon feeding, enrichment distribution, and room cleaning. Temporally predictable husbandry events occurred reliably at the same time daily, while signaled predictable husbandry events were preceded by a distinct event-specific signal in the form of a doorbell. Informal tests prior to study onset found the doorbells not to be aversive to the subjects. Subjects received each of four treatments: unpredictable events, temporally predictable events, signaled predictable events, and temporally and signaled predictable events. Change in stress was evaluated by monitoring changes in motor stereotypies and displacement behaviors. Our results showed that subjects displayed less stress and anticipatory behaviors related to feeding and enrichment events when the events were temporally predictable ($P < 0.0001$). When husbandry events were preceded by a reliable signal, subjects vocalized less prior to the event and were less responsive to activity outside of the room ($P < 0.01$). However this may have come at a cost as the animals were extremely reactive to the doorbell signals and showed a heightened stress response during the actual husbandry events ($P < 0.01$). Similar to temporal predictability alone, when temporal predictability was combined with signaled predictability subjects displayed less stress and anticipatory behaviors related to feeding and enrichment events ($P < 0.0001$). In addition, when both forms of predictability were combined subjects showed less

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stress behaviors while waiting for daily room cleaning ($P < 0.01$). When signaled predictability was paired with temporal predictability subjects no longer had the negative response to the doorbell signal, as they were able to predict and anticipate when the events would occur. Because these results are not necessarily applicable to animals that are given control over their environment or housed in a group setting, the management recommendation that can be made from this study is that temporal predictability of feeding reduces stress and anxiety and is thus beneficial to captive indoor single-housed rhesus macaques.

Keywords

Predictability; Rhesus; Husbandry; Timing of feeding

1. Introduction

Captive animals are frequently faced with the challenge of coping with unnatural environments. Depending on the species and the needs of the institution, these environments can be drastically different from the wild environment to which the animals are adapted. While some species appear to adjust well to captive environments, others show signs of compromised welfare including reduced breeding (Lindburg and Fitchsnnyder, 1994; Wingfield and Sapolsky, 2003), lowered immunity and health outcomes (Dawkins, 1998; Hird et al., 1984), and development of abnormal behaviors (Capitanio, 1986). It is the responsibility of the housing institution and animal caretakers to provide an environment that is best suited for the animals given the needs of the institution and legal and humane requirements.

Chronic exposure to unavoidable stressors is undesirable yet common in many captive primate facilities, and may in part explain the high prevalence of abnormal behaviors (Lutz et al., 2003; Rommeck et al., 2009) and chronic diarrhea (Bailey and Coe, 1999) in rhesus macaques in some facilities. Many common day to day management practices can be potentially aversive to captive macaques, including room cleaning, cage cleaning, and health checks (Line et al., 1991). Past research has shown that rhesus who perceive daily cleaning and health checks as aversive are unable to habituate, despite repeated exposure (Line et al., 1991). The degree to which these events are perceived as aversive can depend on factors such as an animal's ability to control the event, the quality of the human-animal relationship between the animal and caretaker, the presence of a social partner, and the predictability of the event (Bassett and Buchanan-Smith, 2007; Gilbert and Baker, 2011; Kikusui et al., 2006; Minier et al., 2011; Rennie and Buchanan-Smith, 2006). In addition to these aversive events, feeding events can also be a source of stress to some individuals (Waitt and Buchanan-Smith, 2001). Studies have shown that variables associated with increased stress including heart rate, self-directed and abnormal behavior, and stress-related vocalizations increase in the periods prior to feeding in captive primates (Krishnamurthy, 1994; Line et al., 1991; Waitt and Buchanan-Smith, 2001). Husbandry procedures such as room cleaning and feeding occur on a daily basis, and if perceived as aversive or stressful may be a constant source of anxiety and discomfort. It has been theorized that by manipulating the predictability of daily routine events, we can decrease the associated stress and frustration, thus improving animal welfare (Bassett and Buchanan-Smith, 2007).

Predictability can occur in two forms: temporal and signaled (Bassett and Buchanan-Smith, 2007). Temporally predictable events occur reliably at the same time daily, while signaled predictable events are reliably preceeded by a distinct unique signal (Bassett and Buchanan-Smith, 2007). Although temporal and signaled predictability both function to increase the predictability of an event, one should not assume they are equivalent (for a review, see

Bassett and Buchanan-Smith, 2007). Therefore it is always important to test both forms of predictability, rather than assume they will have the same outcome when added to events.

Studies evaluating the effects of predictability on animal welfare have traditionally been conducted on two types of events: aversive events that cause discomfort to the animal, and feeding events. The benefits of adding predictability to aversive events have largely been explained using the “safety signal hypothesis.” This hypothesis states that when an aversive event is reliably preceded by a signal, the absence of the signal communicates to the animal that it is a “safe” period, and the aversive event is not about to occur (Seligman, 1968). Thus, it is expected that a reliable signal before aversive events will reduce stress in captive animals during periods in which there is no aversive event (Lockard, 1963; Seligman, 1968). Similarly, when aversive events are temporally predictable animals know they are “safe” at all other times. Feeding events are not inherently stressful in the same way as aversive events. Rather, the anticipation of feeding events can be a source of stress for captive animals, with inaccurate anticipation leading to frustration (Waitt and Buchanan-Smith, 2001). Such inaccurate anticipation can be unintentionally enhanced by unreliable signals; signals that occasionally occur before an event. A distinct reliable signal prior to feeding events may lessen animals’ dependence on unreliable signals, and effectively communicate what event is about to occur, decreasing frustration (Bassett and Buchanan-Smith, 2007).

Previous research on temporal and signaled predictability have resulted in conflicting and mixed conclusions. While some studies on temporal predictability found it beneficial to make feeding and aversive events temporally predictable (Badia et al., 1975; Krishnamurthy, 1994; Quirce et al., 1981; Ulyan et al., 2006), others found temporal unpredictability to enhance welfare (Bloomsmith and Lambeth, 1995; Hennessy et al., 1977; Jenny and Schmid, 2002; Shepherdson et al., 1993). Research on signaled predictability of aversive events has similarly been inconsistent (for reviews see Badia et al., 1979; Weinberg and Levine, 1980), however research on signaled predictability of feeding events has reliably demonstrated signals before feeding to be a positive welfare tool for captive animals (Carlstead, 1986; Lutz and Perkins, 1960; Prokasy, 1956). With conflicting results on most types of predictability, further research is needed with any species and housing environment before adding predictability to daily husbandry events.

Inconsistencies in previous research may be due to the fact that they have been performed using multiple types of events on a variety of species and environments. Control over one’s environment is extremely important for an animal’s welfare, and previous variations in control and presence of chronic stressors may have affected subjects’ responses to predictability (Bassett and Buchanan-Smith, 2007). Specifically, predictability may be particularly important in environments where animals have limited control, as it provides a means to understand and anticipate an uncontrolled surrounding. Therefore, the specific goal of this study was to identify whether increasing the temporal and signaled predictability of daily husbandry events could decrease stress and anxiety in indoor single housed rhesus macaques.

Temporal and signaled predictability was added to feedings, enrichment distribution, and room cleaning. During the temporal predictability treatment, events happened reliably at the same time daily so animals could anticipate and predict their occurrence. During the signaled predictability treatment, animal staff pressed an event specific doorbell prior to entering a room to perform an activity. Staff may enter an animal room for a variety of reasons, and this can become an unreliable signal that an event is about to take place. The purpose of the doorbell was to remove this unreliable signal and announce which activity was about to occur.

In addition to decreasing the stress associated with the feeding and cleaning events themselves, making events highly predictable may also decrease any stress or frustration associated with husbandry staff entering and exiting rooms at other times of the day. Animals may become frustrated if they incorrectly anticipate husbandry entering a room as a signal that they are going to be fed or cleaned. If feedings and cleanings are on a reliable schedule or preceded by a reliable signal, husbandry staff entering a room at other times or without the signal should inform the animals that no husbandry event is going to occur. To test this, intruder events were performed in which a staff member entered and exited the room at unpredictable times throughout the week.

2. Materials and Methods

2.1. Subjects

The subjects were 14 male and 22 female adult rhesus macaques (*Macaca mulatta*) singly housed indoors in four different rooms at the Oregon National Primate Research Center (ONPRC). The mean age of the subjects was 13.7 years and ranged from 6.6 to 19.7 years. Twenty-seven subjects were reared in outdoor groups, three were mother reared in cages, and six were imported as adults from other facilities with unknown rearing. Rooms were selected based on availability and stability, such that animals were likely to remain in the room for the duration of the study. All subjects were singly housed during the present study for purposes unrelated to this study, in cages 81.3 cm high with 1.4 -2.7 m² of flooring, depending on the size of the animal in accordance with US regulations (Committee, 2011). Monkeys were fed standard monkey chow twice a day (in the morning and afternoon), and were given fresh produce and/or other food enrichment daily. Water was provided freely through automatic lixit systems. The lights were on 12 h per day, from 7:00 h to 19:00 h and the temperature was maintained at 24 ± 2 °C. All subject animals were cared for in compliance with protocols approved by the ONPRC Institutional Animal Care and Use Committee, and participated in the ONPRC Behavioral Management plan. The ONPRC animal care program adheres to the requirements of the Animal Welfare Act from the US Department of Agriculture regulations (Animal Welfare Act, 1985) and is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

2.2. Experimental Design

Subjects received temporal and signaled predictability for four different husbandry events: morning feeding, afternoon feeding, enrichment distribution and room cleaning. Animals did not receive any additional food throughout the day outside of feeding and enrichment distribution. For simplicity, morning feeding, afternoon feeding, and enrichment distribution will be referred to as “food related events” when referenced together. Room cleaning involved a staff member using a hose to clean the floor of the animal room and the base of each animal cage while the animal was still inside. Animals were able to climb on a perch to avoid getting wet. Multiple staff members were responsible for cleaning and feeding different rooms, and the person responsible for a given room varied on a day-to-day basis. This is common practice at the ONPRC and all animals were already familiar with the husbandry staff prior to study onset. On average there were three or four husbandry staff members responsible for any given room. The same staff member usually performed all husbandry activities (e.g., feeding, cleaning, providing enrichment) in a room on a single day, although this was not always the case. Prior to the study onset, staff usually distributed morning feeding between 8:00 h and 9:00 h, and afternoon feeding between 14:00 h and 16:00 h. Enrichment distribution was typically more unpredictable, occurring any time between 10:00 h and 14:00 h. Room cleaning was conducted in the morning at varying times after animals had finished eating their first meal. The specific timing of these activities

varied between the staff and within staff daily. For a detailed description of each event see Table 1.

There were four treatments: unpredictable (U), temporal predictability (TP), signaled predictability (SP), and temporal and signaled predictability (TSP). In treatment U, animals were exposed to the husbandry events at somewhat unpredictable times, which was similar to the normal routine husbandry schedule. To ensure these events did not inadvertently become temporally predictable, husbandry staff recorded the timing of these events, and were instructed not to perform events within 30 min of the previous day's time, but were otherwise not restricted in when they performed the activities. Treatment U was used as a baseline to compare against the predictable treatments. In treatment TP animals received husbandry routines at the exact same pre-determined time every day. In treatment SP, three doorbells, each with a distinct sound, were rigged to the animal rooms. One doorbell was designated for daily AM and PM feedings, one for enrichment distribution, and one for room cleaning. During this treatment, husbandry staff pressed the appropriate doorbell immediately prior to entering a room for these events. Similar to treatment U, husbandry staff performed these events around the same time as normal, but varied them by at least 30 min each day. During the TSP treatment animals received temporal predictability and the doorbell signal prior to cleaning, enrichment, and daily feedings. Commercial General Electric 19209 Wireless Door Chimes were installed in each room as the reliable signals (Jasco Products Company, Oklahoma City, OK, USA). Doorbell sounds utilized in the study include "Westminster," "gong," and "door bell." These specific sounds were selected because they are distinct, and loud enough to be heard over ambient room noise. To avoid any confusion amongst staff the same doorbells were used to represent the same husbandry procedures throughout the study period. Prior to their use in the study, the doorbells were informally tested to see if the sound was aversive to the rhesus. Throughout repeated testing the doorbells did not elicit any stress behaviors and were deemed appropriate for use in the study.

This study was conducted using a repeated measures design, where each room received all four treatments. Order of treatment was balanced using a Latin square design. Each room received each treatment for three weeks, with observations conducted only on week three. With this method, animals had two weeks to learn the new schedule of events before any observations were recorded. In between each treatment, rooms returned to their regular schedule of husbandry activities for one week to avoid carryover effects from the previous treatment.

During the third week of each treatment, animals were subjected to eight "intruder events". These events occurred Monday-Friday, sometimes multiple times in a single day. During an intruder event, a researcher entered the room at an unpredictable time. The timing was unpredictable regardless of the current treatment, however an intruder event never occurred during feeding, enrichment distribution, or cleaning. The intruder stood by the door for 15 s without making eye contact with the animals, and then exited the room.

2.3. Data Collection

Q-See security cameras and security DVR (models QSM1424C8 and QT426) were installed in each room and simultaneously recorded behaviors of all subject animals before and during each husbandry event, as well as during intruder events (Digital Peripheral Solutions Inc., Anaheim, CA, USA). Cameras were real time and recorded video at 30 frames per second. Depending on the distribution of animals in the room, each camera focused on 1-2 animals. A single trained observer, with a test-retest reliability of >85%, analyzed these video recordings. Three types of behaviors were recorded: motor stereotypies, displacement behaviors, and vocalizations (Table 2). Motor stereotypies were recorded using

instantaneous sampling with 12 s intervals, and displacement behaviors and vocalizations were recorded using all occurrence sampling (Altmann, 1974). Motor stereotypes and displacement behaviors were recorded as behavioral proxies of current stress, frustration, and anxiety. Stereotypes are a commonly exhibited abnormal behavior in some populations of captive rhesus macaques (Lutz et al., 2003), and may be defined as repetitive, invariant behavior patterns with no obvious goal or function (Mason, 1991). Motor stereotypes commonly exhibited by macaques include pacing, bouncing, rocking, back-flipping, and swaying. Stereotypes are often used as a measure of well-being, with development and expression of behaviors linked to frustration, lack of stimulation, lack of control, and unavoidable stress (Mason, 1991; Mench and Mason, 1997). Stereotypes are not a perfect indicator of current stress, rate of expression can be viewed as a combination of past neural damage, an index of current frustration, or an attempt to cope (Mason and Latham, 2004). However, once an animal has developed a stereotype, a decrease or increase in rate of expression can be viewed as a direct result of change in either current frustration or current need to cope. Therefore, individual change in stereotype expression can be a useful tool to measure change in individual stress, frustration, and anxiety. Some full body stereotypes seen in captive animals have been hypothesized to be a result of frustration due to failed attempts to forage and seek food (Clubb and Vickery, 2006), and as such change in individual stereotype may be a particularly effective behavioral measure of frustration regarding timing of feeding. Displacement behaviors are behaviors that are functionally unrelated to current activity, and are believed to occur when an animal is in a state of conflict, frustration, uncertainty, or high anxiety (Maestriperi et al., 1992; Schino et al., 1996). Displacement behaviors common to rhesus macaques include yawn, scratch, and shake (Schino et al., 1996). Vocalizations were scored without differentiating among categories of calls because the logistics of that categorization were not practical. Vocalization rate therefore was measured as a proxy for anticipation and reactive behaviors, and was not intended as a direct measure of stress. For a detailed ethogram of behaviors recorded see Table 2.

Observations were recorded 10 min before the event (BE), during the event (DE), and during intruder events (IE). BE observations were conducted exactly 10 min before the event took place. The purpose of BE observations was to determine the level of stress and anxiety in subjects as they waited for events to occur. DE observations were recorded starting when the husbandry member entered the room. For cleaning events, DE observations were conducted for 1 min, during which the staff was prepping the cleaning and usually had not started cleaning individual cages. For food related events, observations were stopped as soon as the subject received food. The purpose of DE observations was to determine the level of stress and anxiety in subjects as the event began. IE observations were taken for 30 s starting when the intruder entered the room. Due to the short nature of IE observations, stereotypes were recorded using 1-0 sampling with 15 s intervals instead of by instantaneous sampling (Altmann, 1974). The purpose of IE observations was to determine the level of stress during unpredictable room activity while other daily events were on a predictable schedule.

2.4. Data Analysis

The data were analyzed using generalized linear mixed effects modeling under the assumption that the underlying response follows a Poisson distribution. Motor stereotypes, displacement behaviors, and vocalizations were treated as outcomes. To determine the effects of predictability condition on stress-related behaviors, data were analyzed with condition as the main effect and individual animal as a random effect. To control for room and order effects, room was also included in the model as a random effect. All analyses of stereotype excluded individuals that were never observed exhibiting stereotype, limiting the

sample to 25 individuals. All individuals vocalized and showed displacement behaviors. Independent models were conducted for each observation type (BE, DE) as well as for “daily feedings,” “enrichment distribution,” “room cleaning,” and “intruder events.” In analyses of daily feedings, the timing of feeding (morning vs afternoon feeding) was included in the model as a main effect. In all analyses the treatment “unpredictable” (unpredictable events) was used as the referent so that results would reflect the effects of the different treatments compared to no predictability. Because all three behaviors recorded were representative of the same construct of stress and anticipation/reaction, a Bonferroni correction was applied and the adjusted alpha value was set at 0.017.

3. Results

3.1. Timing of Events

During the unpredictable timing treatments (U, SP) timing of the events had a standard deviation of ± 52 min. During the temporally predictable treatments (TP, TSP) timing of the events had a standard deviation of ± 4 min. Given the difficulty in coordinating the timing of events in multiple rooms this error was deemed appropriate for the study. During the unpredictable treatments, events happened on average 57 min earlier or later than the previous day. During the temporally predictable treatments, events occurred on average 2 min earlier or later than the previous day.

3.2. Effects of Temporal Predictability

Overall, there was a general decrease in stress and anticipatory behaviors while animals waited for husbandry events that had temporal predictability (BE observations). Subjects were significantly less likely to perform motor stereotypies while waiting for daily feedings and enrichment distribution when these events were temporally predictable, compared to when these events were unpredictable ($\beta = -0.23$, $P < 0.0001$; $\beta = -0.61$, $P < 0.0001$; Fig. 1). Subjects were also significantly less likely to vocalize while waiting for daily feedings, enrichment distribution, and room cleaning, when these events were temporally predictable, compared to when they were not predictable ($\beta = -0.30$, $P < 0.0001$; $\beta = -0.40$, $P < 0.0001$; $\beta = -0.83$, $P < 0.0001$; Fig. 1).

Similarly, there was also a decrease in stress and reactive behaviors during feedings (DE observations) that were temporally predictable. Subjects were significantly less likely to perform motor stereotypies and vocalize during daily feedings that were temporally predictable ($\beta = -0.400$, $P < 0.0001$; $\beta = -0.58$, $P < 0.0001$; Fig. 2).

There were no significant behavioral changes in response to the unpredictable intruder events (IE observations) when husbandry events were temporally predictable.

3.3. Effects of Signaled Predictability

Overall there was a decrease in vocalizations while animals waited for husbandry events with signaled predictability. Subjects were significantly less likely to vocalize while waiting for daily feedings, enrichment distribution, and room cleaning when these events were preceded by a reliable signal, compared to when they were unpredictable ($\beta = -0.29$, $P < 0.0001$; $\beta = -0.26$, $P < 0.01$; $\beta = -0.76$, $P < 0.0001$; Fig. 1). However, there was not a consistent change in stress-related behaviors while animals waited for husbandry events with signaled predictability. Subjects were both significantly *less* likely to perform motor stereotypies and significantly *more* likely to perform displacement behaviors while waiting for enrichment distribution when it was preceded by a reliable signal ($\beta = -0.36$, $P < 0.0001$; $\beta = 0.34$, $P < 0.01$; Fig. 1).

Overall there was a consistent increase in stress and reactive behaviors during food related events that were preceded by a reliable signal. Subjects were significantly more likely to perform motor stereotypies during enrichment distribution ($\beta = 0.67$, $P < 0.0001$; Fig. 2), more likely to perform displacement behaviors during enrichment distribution ($\beta = 1.14$, $P < 0.01$; Fig. 2), and more likely to vocalize during daily feeding and enrichment distribution ($\beta = 0.43$, $P < 0.001$; $\beta = 1.20$, $P < 0.001$; Fig. 2) when these events were preceded by a reliable signal.

When husbandry events were preceded by the reliable signal, subjects were significantly less likely to vocalize during the unpredictable intruder events ($\beta = -1.31$, $P < 0.01$; Fig. 3).

3.4. Effects of Temporal and Signaled Predictability Combined

Similar to temporal predictability alone, there was an overall decrease in stress and anticipatory behaviors while animals waited for husbandry events that had temporal and signaled predictability. Subjects were significantly less likely to perform motor stereotypies while waiting for daily enrichment distribution and room cleaning when these events were temporally and signaled predictable, compared to when these events were unpredictable ($\beta = -0.78$, $P < 0.0001$; $\beta = -0.26$, $P < 0.01$; Fig. 1). Subjects were also significantly less likely to vocalize while waiting for daily feedings, enrichment distribution, and room cleaning when these events were temporally and signaled predictable ($\beta = -0.54$, $P < 0.0001$; $\beta = -0.47$, $P < 0.0001$; $\beta = -1.42$, $P < 0.0001$; Fig. 1).

Similar to temporal predictability alone, there was a decrease in stress-related behaviors during feedings that were temporally and signaled predictable. When made temporally and signaled predictable, subjects were significantly less likely to perform motor stereotypies during daily feedings ($\beta = -0.31$, $P < 0.001$; Fig. 2). Unlike signaled predictability alone, there were no significant increases in stress and reactive behaviors during food related events.

There were no significant behavioral changes in response to unpredictable intruder events when husbandry events were temporally and signaled predictable.

4. Discussion

4.1. Temporal Predictability

Overall, there was a decrease in stress and anticipatory behaviors while animals waited for food related events with temporal predictability. Similarly, there was a decrease in stress and reactive behaviors during feedings that were temporally predictable. Taken together, it appears that making food related events temporally predictable is an effective way to decrease the stress associated with both waiting for and experiencing these events.

Surprisingly, this finding is contrary to the common conception that it is problematic to feed captive animals on a predictable schedule (Bassett and Buchanan-Smith, 2007; Buchanan-Smith et al., 2009). Because anticipation of food is believed to be a source of stress, it has previously been hypothesized that highly predictable timing of food distribution increases anticipation and thus increases individual stress. We suggest that despite increasing anticipation, a predictable schedule of events also effectively removes the “unknown” of feeding. Without temporal predictability animals are in a constant state of unknown as to when their next feeding will occur. With temporal predictability this unknown is removed and animals can accurately predict when food will arrive. Although this knowledge may build anticipation, our results indicate that the stress of this anticipation is less than the stress of not knowing when food will arrive. The beneficial effect of knowing when food was

arriving was so strong that monkeys were less reactive and displayed less stress-related behavior during the feeding event itself.

In addition to the beneficial effects of temporal predictability on food related events, there was also a decrease in vocalizations before cleaning events. Unfortunately the method of data collection did not allow us to determine if this change was due to a decrease in alarm calls or a decrease in food vocalizations. It is therefore possible that these changes in vocalizations were simply an artifact of the predictable daily feedings, and not related to the predictable cleaning. When husbandry events had no temporal predictability, enrichment distribution would occasionally happen before cleaning events. With temporal predictability, cleaning events always preceded enrichment distribution. Therefore, the decrease in vocalizations prior to cleaning may be a decrease in food vocalizations because the monkeys were no longer anticipating enrichment distribution. Because the study design did not allow for cleaning events to be temporally predictable while daily feedings were temporally unpredictable, it is impossible to fully separate the effects of each event on the other. Similarly, we can not be sure if the beneficial effects of temporal predictability related to enrichment distribution were due to the consistent timing of the event, or the consistent order of the event (although this does not impact our results on feeding, which were always the first and last event of the day). Future research is needed to determine if it is more important to have events happen at a specific time, or simply in a specific order.

4.2. Signaled Predictability

Animals were less likely to vocalize while waiting for husbandry events when the events had only signaled predictability. The husbandry events were still temporally unpredictable, so the animals were unable to predict *when* the events were going to occur. However, because an event specific doorbell always preceded the events, this doorbell became a reliable signal that the animals could listen for immediately prior to the events. We believe that the high number of anticipatory vocalizations prior to events with no signaled predictability were in response to the numerous unreliable signals for each event. For example, when staff provides morning and afternoon feedings they bring the food on a rolling cart. The sound of this cart moving down the hall outside of the room is a signal that the animals may soon be fed. However, carts may be moved in a hall for reasons unrelated to food, so this sound is an unreliable signal. Similarly the voices of staff members outside rooms or nearby doors opening and closing may be unreliable signals for any given event. With no reliable signal prior to events, the animals may be more likely to respond to unreliable signals with vocalizations. When given a reliable signal in the form of a doorbell, the animals could learn to listen only for the doorbell, and ignore the unreliable signals.

There was also a decrease in vocalizations during the unpredictable intruder events when husbandry events had signaled predictability. It is likely that the animals were less responsive to the human intruder because the absence of the doorbell prior to the intruder entering the room effectively communicated that the intruder was not coming in to feed or clean. In contrast, during the unpredictable treatment a staff member entering a room might have been an unreliable signal to the animals that they were going to be fed, thus prompting food vocalizations.

Although the macaques were cuing into the doorbell signals and were less responsive to unreliable signals, it is unclear if this was effective in decreasing stress and increasing the welfare of the animals. While signaled predictability decreased motor stereotypies prior to enrichment events, it also increased displacement behaviors. Unfortunately, these conflicting results make it difficult to reach any conclusions about the welfare implications of signaled predictability during the periods prior to husbandry events.

However, regardless of the effects of signaled predictability on animal welfare while waiting for husbandry events, our results found a clear negative consequence of reliable signals during the actual food related events. Once staff pressed the doorbells and entered the room to distribute food or enrichment, there was an increase in stress and reactive behaviors, compared to the unpredictable treatment. Thus, although the reliable signal allowed the animals to disregard unreliable signals, the signals themselves unintentionally amplified the negative behaviors we were aiming to reduce. This result is surprising, given past research that has consistently demonstrated the positive effects of signaled predictability for feeding events (Carlstead, 1986; Lutz and Perkins, 1960; Prokasy, 1956). This leads to unanswered questions as to why signaled predictability was not beneficial in this study. It is possible that although early tests found the doorbells to be unobtrusive, the sound of the doorbells were indeed aversive to the animals. Future research is needed to determine if using different signals would change the outcome of the present study, or if signals are simply not beneficial for rhesus macaques in this specific environment.

4.3. Temporal and Signaled Predictability

When temporal and signaled predictability were combined, the resulting change in behavior was similar to temporal predictability alone. Like temporal predictability, subjects receiving temporal and signaled predictability showed a decrease in stress and anticipatory behaviors while waiting for food related events. However, a benefit only seen with temporal and signaled predictability combined was the significant decrease in stereotypy before cleaning events. Cleaning events were different from the other observed husbandry events in that they are believed to be aversive to the animals. It therefore appears that for aversive events it may not be enough to utilize only one form of predictability. Rather, for rhesus macaques, the aversive event cleaning was only significantly less stressful when the subjects were able to both anticipate the timing of the cleaning and ignore unreliable signals via the utilization of a reliable doorbell signal.

What is perhaps most surprising is that subjects showed less stress-related behavior during daily feeding, and showed no increase in vocalizations during any of the husbandry events that had temporal and signaled predictability. This is surprising because there was a large increase in stress-related behaviors and vocalizations during food related events that had signaled predictability alone. Thus it appears that the addition of temporal predictability to the reliable signal made the actual events less stressful, and successfully removed the negative response to the doorbell. As previously mentioned, it is possible that the building anticipation for food related events makes the events a source of stress for the animals. When food related events occur at unpredictable times, the uncertainty about when these events are going to happen presumably fuels the stress and anticipation of the animals. With the animals already in a high state of stress and anticipation, doorbells signaling onset of the feeding may result in a particularly pronounced reaction. With the temporal and signaled predictability treatment the animals expected the food to arrive at a particular time, so there was not the same amplified reaction to the doorbell. Therefore, temporal predictability may be a necessary addition to signaled predictability in order to remove dependence on unreliable signals and avoid the negative amplification of problem behaviors. Alternatively, overshadowing of signaled predictability by temporal predictability may explain why animals responded to temporal and signaled predictability combined in a similar fashion to temporal predictability alone. If temporal predictability is a more salient predictor for rhesus, they may only cue in on the timing of events and ignore the reliable signals altogether. This would explain why temporal and signaled predictability combined had positive effects similar to temporal predictability alone without the negative effects of signaled predictability. However, since adding signaled predictability to temporal

predictability complicates the husbandry routine without providing a clear benefit to the animals, temporal predictability alone may be a more practical means of animal care.

4.4. Limitations

To our surprise, there was little change in behavioral response to the intruder events during the different predictability conditions. We expected the subjects to show a stress or frustration response during intruder events in the unpredictable treatment because the intruder opening the door and entering the room may signal the beginning of a food or cleaning event. In contrast, during the signaled and temporal predictability treatments, the absence of a doorbell and the timing of the intruder event should have signaled to the animals that no husbandry event was going to occur. We believe that the lack of results may have been due to the fact that only one researcher conducted all intruder events. This researcher never interacted with the animals, and it is possible that the macaques were quickly able to learn that the intruder was not going to feed the animals or clean, and thus were generally unresponsive to this intruder. Future research including intruder events should utilize multiple intruders, ideally those who have a history of interacting with the subjects. Further research should also consider conducting observations at multiple time points during the day. This study specifically investigated the stress of the animals waiting for events 10 min before the event occurred. Although this was adequate to find significant decreases in stereotypy, it did not address how overall levels of stress and anxiety were affected at all other times of the day. It would be useful to know if temporal predictability of feeding was able to decrease overall levels of stereotypy at times of the day other than prior to and during feeding.

Finally, we would also like to point out that the observed changes in stress-related behaviors were not always consistent across all individuals in the study, indicating that when some animals benefited others might not have. Individual differences between rhesus macaques, arising from differences in rearing condition and temperament, can help account for differences in development and expression of motor stereotypy (Vandeleest et al., 2011), and may explain why not all individuals showed the same response to the different treatments. Although different treatments and schedules may be optimal for different individuals, this issue becomes difficult to resolve with events such as cleaning and feeding that affect all individuals at the same time. It is also important to note that most of the significant changes in behavior were seen in stereotypy and not displacement behaviors. Not every animal displayed stereotypy (25 out of the 36 displayed the behavior), and therefore these results do not necessarily represent the responses of the remaining 11 animals. Future research is needed to understand how to best optimize husbandry schedules for all animals, taking into account individual differences and needs while remaining practical from a management perspective.

4.5. Implications

The outcome that was most consistent across all groups and conditions was the beneficial effects of temporal predictability on food related events. This is also one of the most surprising results, as many previous studies have found beneficial and enriching effects of feeding captive animals at unpredictable times (Bloomsmith and Lambeth, 1995; Jenny and Schmid, 2002; Shepherdson et al., 1993). We believe our results are so strikingly dissimilar from these previous studies because the indoor captive laboratory primate environment can be vastly different from other captive environments. Due to the restrictions and current practices of many research facilities in the US, indoor-housed rhesus often have less stimulation than outdoor and group housed animals, and less control over their environment. In this study, all animals were singly housed in a caging appropriate for their size. In this restricted environment it appears that the “unknown” associated with unpredictable feeding

is extremely stressful and problematic, and outweighs any potentially enriching effects of randomized feeding. Adding a predictable schedule of events may help restricted animals understand and cope with an otherwise uncontrollable environment. Thus, while unpredictable feeding may be suitable for animals socially housed or in large zoo enclosures, this study has found predictability to decrease stress in extremely restricted captive environments.

However, it should be noted that much of the previous evidence for enriching effects of temporal unpredictability have been confounded by additional factors such as increased foraging opportunities (Jenny and Schmid, 2002), increased control (Shepherdson et al., 1993), or lack of a true “random” treatment (Waitt and Buchanan-Smith, 2001; but see Bloomsmith and Lambeth, 1995). Much of the argument against temporal predictability of feeding comes from observations that stereotypy and other stress behaviors increase prior to scheduled feedings (Carlstead, 1995; Krishnamurthy, 1994; Weller and Bennett, 2001). Yet this alone does not imply that unpredictable feeding schedules will decrease these negative behaviors, as unpredictable feeding schedules may simply increase the period of undesirable anticipation. In a recent study on socially housed capuchins, Ulyan et al. (2006) found unpredictable feeding schedules to decrease animal welfare. As one of the few studies creating a true unpredictable feeding schedule treatment, Ulyan et al.'s (2006) findings provides further evidence along with this current study that unpredictable feeding schedules *alone* may not be conducive to higher welfare, regardless of social environment. Further research is needed on additional species and environments to determine if and when random feeding schedules alone can be beneficial.

In conclusion, the beneficial effect of temporal predictability for food related events is an exciting find for laboratory primate management, especially for single-housed populations with limited control and social support. Single housing is a last resort in primate housing that is only acceptable under very specific circumstances, and in these cases it is especially important to find means to improve animal welfare. This research has successfully demonstrated that a simple change in timing of husbandry events can decrease daily animal stress.

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Fig. 1.

Effect of Predictability Before Husbandry Events. Predicted percent change in behavior in animals waiting for husbandry events with predictability, compared to husbandry events with no predictability. Note: Asterisks indicate significant differences at $P < 0.017$.

Fig. 2. Effect of Predictability During Husbandry Events. Predicted percent change in behavior in animals during husbandry events with predictability, compared to husbandry events with no predictability. Note: Asterisks indicate significant differences at $P < 0.017$.

Fig. 3. Effect of Predictability on Intruder Events. Predicted percent change in behavior during intruder events that occurred when husbandry events were predictable, compared to intruder events when husbandry events were random. Note: Asterisks indicate significant differences at $P < 0.017$.

Table 1

Description and average time of daily husbandry events to which temporal and signaled predictability were added

Event	Average Time of Event	Event Description
Morning Feeding	8:00 – 9:00 h	Staff member enters room with cart filled with monkey chow. Staff member hand distributes monkey chow into the floor or top of each cage.
Room Cleaning	9:00 – 12:00 h	Staff member uses a hose to clean the floor of the animal room and the base of each animal cage while animal is still inside.
Enrichment Distribution	9:30 – 14:00 h	Staff member enters room with or without cart. Staff member hand distributes produce enrichment into the floor or top of each cage in the room. Enrichment varies from day to day.
Afternoon Feeding	14:00 – 16:00 h	Staff member enters room with cart filled with monkey chow. Staff member hand distributes monkey chow into the floor or top of each cage.

Table 2

Ethogram of behaviors recorded before and during husbandry events

Behaviors scored as motor stereotypies	Pacing	Walking in the exact same pattern – either back and forth or in a circle – 3 or more repetitions
	Swinging	Grasping a part of the cage with one or more hands or feet while moving in the exact same pattern – either back and forth or in a circular pattern - for 3 or more repetitions
	Flipping	3 or more repeated forward or backwards somersaults
	Twirling	3 or more repeated 360° turns of the body while standing in place
	Bouncing	Jumping up and down for 3 or more repetitions using a rigid posture. This behavior should not be confused with bouncing with a less rigid posture, which appears to serve only to make noise or shake the cage
	Swaying	Rhythmic movement either side to side or forward and backward for at least 3 repetitions
Behaviors scored as displacement behaviors	Yawn	Slowly opening the mouth to a wide position, exposing the teeth
	Scratch	Vigorous strokes of the hair and/or skin by the fingernails or toenails
	Body Shake	Shaking of the entire body similar to that of a wet dog
Other behaviors recorded	Vocalize	Any sound produced by the mouth