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Reliably signalling a startling husbandry event improves welfare of zoo-housed capuchins (*Sapajus apella*)

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## **Abstract**

Animals kept in captivity are reliant on humans for their care and welfare. Enclosure design, and choice of group mates as well as routine husbandry events such as feeding, cleaning, and health care are in the hands of human keepers. It is therefore important to understand how external human-related husbandry events affect daily behaviour routines for animals, to help promote good welfare.

Predictability (or lack thereof) of these routines can have profound effects on behaviours of captive animals. This study investigates whether providing a reliable predictable signal indicating entry into indoor brown capuchin (*Sapajus apella*) enclosures can increase welfare. All day focal follows of 12 zoo-housed capuchins were performed, recording behaviour in relation to husbandry events. The Baseline data show that unreliable sounds of door openings and closings outside the enclosure increase anxiety-related behaviours such as self-scratching, vigilance and jerky motions, and that the capuchins were startled by keepers entering the enclosure. A reliable signal (knocking) was subsequently introduced before enclosure entry and the monkeys given two weeks to associate the signal prior to Treatment condition data collection. The results indicate that the anxiety-related behaviours were reduced in the Treatment condition compared to Baseline frequencies. We conclude that making certain husbandry events reliable and predictable through the introduction of a unique signal can have a significant positive impact on the welfare of animals. Such an approach is not time consuming and costs nothing to implement, yet can result in significant advancements in animal welfare that can be implemented in a wide range of captive settings.

**Key words:** Predictability, signal, welfare, capuchin, *Sapajus*, *Cebus*

## 1. Introduction

Efforts are constantly made to improve the welfare of animals in captivity. These include improving physical and social housing conditions, increasing sensory stimulation, providing opportunities for cognitive challenge, feeding enrichment and improving the human-animal relationship. Whilst many of these environmental enrichment attempts meet with good success, they are often time consuming, and financially expensive limiting their implementation. One aspect of keeping animals in captivity that is largely overlooked is the predictability of events, and how manipulations of predictability can positively impact on animal welfare.

Understanding animal feelings, or emotions, is central to welfare assessment and efforts to improve their welfare. Although naturalistic (e.g. Bracke and Hopster, 2006) and biological functioning (e.g. Broom and Johnson, 1993) approaches to welfare assessment still play critical roles, the focus has moved to the individual animal's subjective state (e.g. Dawkins, 1988; Mendl et al., 2010). Theories suggest that animal emotions stem from how they assess situations (Paul et al., 2005). This assessment is influenced by many factors, including the intrinsic properties of the situation (including its predictability, familiarity, and suddenness), its importance in relation to the individual's current needs, the individual's perceived valence of the situation (aversive/pleasant) and intensity, and its resilience or coping abilities. One such factor, predictability, is investigated in this research which quantifies the effects on welfare of changing predictability of an event that animals anticipated with increased anxiety-related behaviours. The findings are interpreted in relation to valence and suddenness.

It is well known that the predictability of an event affects an animal's response to it (e.g. Weinberg and Levine, 1980; reviewed in Bassett and Buchanan-Smith, 2007). Early experiments investigating the effects of predictability of stimuli on animal behaviour and welfare have manipulated "temporal" and "signalled" predictability (Bassett and Buchanan-Smith, 2007). The former manipulates temporal characteristics of the stimulus presentation (i.e. delivering it to the animal on either a fixed-

time or variable-time schedule). For example, a positive stimulus, such as food, or an aversive stimulus, such as electric shock, might be delivered to animals at random times, which are irregular and therefore unpredictable. Alternatively, the stimulus could be delivered at fixed times, which are regular and therefore predictable. The second method involves preceding the stimulus with a signal. A regular signal preceding the stimulus by the same time interval will render it predictable, irrespective of whether it occurs on a fixed or variable time schedule. Variations in the predictability of the stimulus may be achieved by manipulating the reliability of the signal preceding it. Thus, a stimulus occurring after 50% of signals will be less predictable than one occurring after 100% of signals. Bassett and Buchanan-Smith's (2007) review, together with recent experimental work (e.g. Greiveldinger et al., 2007) strongly suggests that signalled predictability is more salient to animals than temporal predictability (e.g. Badia et al., 1975). Although control and predictability are inextricably linked, that is if the animal has control over a stimulus it will be able to predict its occurrence (Bassett and Buchanan-Smith, 2007; Buchanan-Smith and Badihi, 2012), this research focuses on husbandry events which animals learn to predict (through classical conditioning) that are out of the animal's control.

A number of behavioural studies have used electric shocks as aversive stimuli, and rendered the signalled predictability of these events high (by reliably preceding their presentation by a conditioned stimulus acting as a signal, e.g. a tone or light) or low (no signal). Rats, fish and birds offered a choice between predictable and unpredictable electric shock will generally choose shock with high signalled predictability (reviewed by Badia et al., 1979), whether or not they are able to escape it. Predictable shock has also been found to be less behaviourally disruptive than unpredictable shock (e.g. Davis and Levine 1982). These results have been taken to suggest that an element of predictability makes shock less aversive. This effect is so pronounced that rats chose signalled rather than unsignalled shock even when the predictable shock was four to nine times longer and two to three times stronger than that in the unpredictable condition (Badia et al., 1973).

Although the findings of effects of predictability on physical and physiological measures are less consistent, they come primarily from temporal predictability (as opposed to the more salient signalled predictability) and they also suggest that aversive events should be made predictable (reviewed in Bassett and Buchanan-Smith, 2007). Such a conclusion is supported by a variety of studies showing that unpredictable environmental conditions result in elevated concentrations of corticosteroids in a range of vertebrates, including mammals, birds, reptiles, amphibians and teleost fish (review by Wingfield and Ramenofsky, 1999). The long-term activation of these hormones is unlikely to be adaptive in a free-living individual, as it may result in delay of puberty, suppression of growth, metabolic exhaustion from breakdown in skeletal muscle, increased susceptibility to disease and neuron death in the hippocampus (Wingfield and Ramenofsky, 1999).

The perceived valence of the event will affect the type of predictability that best promotes welfare (Bassett and Buchanan-Smith, 2007). There are a number of aversive events that occur in the life of captive animals. Common aversive stimuli might include cage cleaning, or catch up for veterinary intervention and providing a reliable signal to indicate the onset of an aversive event (and thus the absence of such a signal indicates that the situation is safe) should improve welfare (Bassett and Buchanan-Smith, 2007). Given that it is often the same staff that will perform neutral, positive and negative events (such as animal checking, feeding, and catch up), the staff may become an unreliable signal to any such events. One negative association is often sufficient to induce fear and animals have long memories, especially for those associated with negative experiences (Mendl et al., 2001). The use of a unique signal to indicate a potentially anxiety-inducing event may therefore be beneficial.

There are several studies which strongly support this hypothesis. For example, in humans, Fonteyne et al. (2009) showed that chronic anxiety was reduced by the introduction of a visual cue to signal an aversive event. Using a within subjects experimental design, they conditioned to a cue using predictable electric shocks, or conditioned to a context using unpredictable shocks. The results (startle response and

self-rating of expectancy of the shock) clearly demonstrated that context conditioning is reduced when a discrete cue that predicted the onset of an aversive event is added. In relation to animals in captivity, this finding suggests that animals can learn to ignore unreliable context cues that may lead to chronic anxiety.

Greiveldinger et al. (2007) used a similar paradigm of signalling a sudden, startling event whilst lambs were feeding. They showed that compared to unpredictable (no signal) or temporally predictable sudden events, those lambs with a predictable (light) signal responded less. Measures of suddenness-induced startle, and cardiac response, were decreased, whilst feeding was increased in the predictable condition, suggesting that not only is the sudden event less aversive, but also that animals are better able to relax at other times. These findings are explained by the 'safety-signal hypothesis' which states that if an event is predicted by a signal, the absence of that signal indicates that the situation is safe, and that no such event will occur (e.g. Seligman, 1968). When events are predictable, animals will be in a state of anticipation/readiness only when the signal is present, and not in its absence. Seligman and Meyer (1970) claim that this hypothesis explains the negative physiological and behavioural effects of unpredictability of aversive events.

Previous work on predictability has focused on non-ecologically relevant aversive events (electric shocks) and predictability of appetitive events (food). It is generally accepted that positive events such as feeding should be on a temporally unpredictable schedule to reduce unwanted anticipatory and abnormal behaviours (e.g. Carlstead, 1998; reviewed in Bassett and Buchanan-Smith, 2007). However one study on capuchins found that when a temporally predictable feeding schedule was switched to be unpredictable, behavioural signs of poor welfare increased, as did cortisol levels (Uliyan et al., 2006). This may have been because the capuchins were still anticipating food on a temporally predictable schedule that was often delayed, which is known to be poor for welfare (Waitt and Buchanan-Smith, 2001). This current research focuses on how animals respond to routine husbandry

events, and manipulates the predictability of a startling event using a signal, and hence has broad applicability.

Before altering the predictability of an event it is important to understand whether the animals perceive the event as positive or negative. This can be done by assessing behavioural responses to an event in order to determine whether there is justification for change. By observing and analysing daily behaviour patterns of captive brown capuchin monkeys (*Sapajus apella*) and linking those behaviours with external stimuli (which the animals have no control over), it is possible to determine which aspects of daily husbandry, if any, negatively affect welfare and those which are positive (as one might expect for feeding). Events which have been determined to be startling or aversive can be made predictable by the implementation of a reliable signal, allowing the affected animals to mentally and physically prepare themselves, alleviating possible stress, related to anticipation of a perceived negative event. Moreover, implementation of a signal which is simple, inexpensive (free in this case!) and improves welfare could be an extremely useful technique for use in other institutions which house captive animals.

### **1.1 Assessment of Welfare**

Welfare assessment is complex, and should ideally combine a variety of measures, including physical, physiological and behavioural (Mason and Mendl, 1993). However, this research will focus on behavioural welfare assessment, as behaviour is the most accessible and practical measure of welfare, and one of the most informative of welfare state. Behaviour is the ultimate phenotype – not only is it the result of all of the animal's own individual decision making processes it is also the expression of emotions (Darwin, 1872; Dawkins, 2004). Behaviour provides an immediate and quantifiable measure to assess welfare state (unlike some physiological, cardiovascular and haematology and clinical chemistry measures) and as Dawkins (2004) notes, it is non-invasive (e.g. does not require breaking the skin), and in many cases also non-intrusive (the animal may not be disturbed in any way). Given the excellent



background literature on capuchin behaviour and the contexts in which they occur (Fragaszy et al., 2004; Freese and Oppenheimer, 1981), welfare was assessed by recording behaviour.

## 1.2 Hypotheses

Based on the literature and *ad libitum* observations, the following two hypotheses are made:

- (1) Unreliable signals indicating key husbandry events negatively impact on captive capuchin behaviours.
- (2) Making a startling husbandry event more predictable via reliable signalling will decrease anxiety-related behaviours shown in anticipation of the event.

## 2. Methods

This project uses a data-rich observation technique (all day focal sampling) in order to assess the responses of capuchins to husbandry routines. By plotting these all day focal samples it is possible to determine how an animal perceives external events, and then to alter the predictability to monitor impact.

### 2.1 Subjects and Housing

Individuals observed for this study were housed at the Living Links to Human Evolution Research Centre located in the Royal Zoological Society of Scotland's (RZSS) Edinburgh Zoo, Edinburgh, Scotland, UK. The building houses two mixed-species groups ('west' and 'east') of brown capuchin monkeys (*Sapajus apella*, formerly known as *Cebus apella*, Alfaro et al., 2012) and common squirrel monkeys (*Saimiri sciureus*) in mirror image enclosures. Each mirror image side consists of an indoor squirrel monkey enclosure (5.5m x 4.5m x 6m high not accessible to the capuchin monkeys), an indoor capuchin enclosure (7m x 4.5m x 6m high, accessible to the squirrel monkeys), a testing room for cognition research and training (providing an access route between the indoor and outdoor enclosures through a 2m link of cubicles), a shared outdoor enclosure (approximately 900m<sup>2</sup>) and off-show holding areas that are used for husbandry and veterinary reasons for each of the four species groups (~ 12m<sup>3</sup>; Figure 1 and

photographs in MacDonald and Whiten 2011 and on <http://www.youtube.com/watch?v=BazrVgfMCvI>). Indoor enclosures have bark chips on the floor, a complex network of branches and lianas, artificial rock areas and wooden feeding platforms, and several windows providing natural light. The ground of outdoor areas is a mixture of bark chip, grass, soil and live shrubbery. As there is no roof, there is a network of trees located centrally (to allow for a possible 7m jump zone). There is a 2.4m high perimeter mesh fence with electric hot wires at the top. The public can view the monkeys and into the research rooms from a 3m high walkway that runs through the centre. Underneath this walkway, there is keeper access, researcher offices and accommodation (further details in Leonardi et al., 2010; MacDonald and Whiten, 2011).

In order to access the indoor capuchin enclosures from outwith the facility, keepers must open three doors in total. The first one leads to the keeper access area, the next leads to a small safety area (preventing any escapees) and the third leads into capuchin enclosure,. There are several other doors, which are also audible when opened (see Figure 1), that lead to the squirrel monkey enclosures and provide the capuchins with cues to keeper activity. Monkeys had constant access to both indoor and outdoor enclosures and to the testing areas except for brief periods during this study.

Figure 1 about here

Twelve capuchins were studied: six individuals from the west group (which consisted of 12 capuchins in total: three adults males, three adult females and five young males and one young female < 4 years) and six individuals from the east group (nine capuchins in total; four adult males, three adult females and two young males). Individuals from the east and west group were matched as closely as possible based on sex, age and rank (see Table 1). Observations were made (by KR) on the three days each week when no cognitive testing was performed during May-August 2010. Observations were carried out from public viewing areas and data scored on check sheets.

Table 1 about here

## 2.2 Husbandry Routines

Indoor enclosures receive a basic clean every day, between 8:30 and 10:00, which consists of removal of food from the day before as well as spraying down surfaces and windows with water. Once a week, enclosures receive a thorough clean, including washing of all windows, walls, and surfaces with soap and water. Food is distributed throughout the day: following the morning enclosure clean (between 9:00 and 10:00), before the keepers take lunch (around 13:00), late in the afternoon (between 15:30 and 16:00), and a final evening feed (around 16:45). Dietary value and quantity of food delivered is dependent on enrichment, training, and testing sessions in order to provide optimal nutritional balance without overfeeding. Depending on weather conditions, any or all of the feeds may be distributed inside or outside. If distributed outside, the food was thrown from the viewing deck (see Figure 1) and did not involve entry to the enclosure. If distributed inside the keepers entered the indoor enclosure. Usually the capuchins would come to eat quickly when the keeper was still present in the enclosure although some capuchins waited for the keeper to leave the enclosure. Water was available at all times *ad libitum*. Further details of the Living Links centre and housing and husbandry are provided in Leonardi et al. (2010) and MacDonald and Whiten (2011).

All of the husbandry routines occur on a relatively temporally predictable schedule (although feeding is unpredictable in delivery location) but all are unreliably signalled by various sounds. From entering the keeper area, a minimum of three doors need to be opened before a keeper enters the capuchins' internal enclosure, and any activity in the keeper area and sounds of doors opening and closing could be used by the animals as a cue to the occurrence of any of the aforementioned husbandry events. The sound of doors would be a reliable signal if they always indicated a keeper's entry to their enclosure, but, as keepers can pass through doors leading to the squirrel monkeys, or going to the capuchin holding area without entering the indoor capuchin enclosure, they are not. Very occasionally keepers would have to enter either the indoor or outdoor enclosure to retrieve an object dropped by

the public, and they engaged in other activity in the keeper area, such as sweeping, moving food to stores etc. With this in mind, knocking on the one internal door immediately leading to the capuchin enclosure was chosen as a signal for keeper entry in to the capuchin enclosure, which can be sudden and startling for them. This signal is a reliable indicator of entry and is only heard by the capuchins when a keeper enters their indoor enclosure. By introducing this unique reliable signal, the capuchins should disassociate the unreliable signal of other door openings with possible entry to the indoor enclosure.

### **2.3 Baseline condition**

One complete day of baseline data was collected between 08:15 and 17:01 for each individual included in the study via continuous focal sampling (Altmann, 1974; Martin and Bateson, 2007), and frequencies of behaviours adjusted for time out of sight in analyses. A number of potentially anxiety-related behaviors were recorded: autogrooming, threat faces, branch shaking, urine washing, self-scratching, vigilance and jerky motion (Table 2 gives definitions of behaviours reported). Short breaks (<10 min) from observation were taken when it was known that keepers were not active. Sampling of individuals alternated between east and west groups.

Table 2 about here

### **2.4 Preliminary analysis to justify implementation of signalling**

Before introducing a reliable signal, data collected in the Baseline condition were analysed in order to determine whether any events were anticipated negatively. All individuals were analysed separately, and as an entire group as we are aware of individual differences amongst capuchins, and considered that husbandry routines may impact individuals differently, in relation to rearing history, previous interactions with humans etc. Frequencies of potentially anxiety-related behaviours were recorded, namely scratching, vigilance, autogrooming, jerky motions, branch shaking, threat faces, and urine washing. These were plotted over time and in relation to husbandry events with resulting graphs used to determine how baseline analysis would proceed.

For baseline analysis, data were divided into 5 minute “before door event” and 5 minute “after door event” categories. A door event was defined as the opening and/or closing of any door in the keeper area which was clearly audible as they are metal. Door events which followed another door event with less than a 10 minute interval were classified as a ‘sequence,’ and only the 5 minutes before and after the first door in the sequence were analysed. Door events separated by more than 10 minutes were treated as independent events.

Preliminary analyses showed three potentially anxiety-related behaviours were significantly higher (or showed trends,  $P < 0.06$ ) after door events compared to before door events, regardless of human entry to the internal enclosure. These were scratching, vigilance and jerky motions (Wilcoxon Signed rank tests). Other behaviours recorded did not show a consistent pattern ( $P > 0.06$  for all). These results justified the choice of knocking on the internal door before entry into the enclosure as the reliable signal for the Treatment condition.

## **2.5 Treatment condition**

The signal of reliable knocking was introduced and consistently performed by all keepers who were reminded by a sign placed on the internal capuchin door. The knocking occurred three seconds before keeper entry. The capuchins were given two weeks to learn to associate the signal before data collection proceeded. One full day of focal sampling per individual was performed using the same method as Baseline data collection.

## **2.6 Data Analysis**

Data were tested for normality using Kolmogorov-Smirnov test, with each behaviour analysed separately for normality. No behaviours were normally distributed and attempts to normalize using a variety of transformation techniques failed, so non-parametric tests were used. A Wilcoxon signed rank test was used to compare behaviours before and after a door event. Friedman’s ANOVA was used to identify significant differences between conditions, with *post-hoc* Wilcoxon signed rank test. Bonferroni

corrections were made for multiple tests ( $n=6$ , therefore  $P<0.0083$ ). Multiple Friedman's ANOVAs were performed as a non-parametric replacement for two-way ANOVA, with *post hoc* analysis as above.

### 3. Results

#### 3.1 Hypothesis 1: Unreliable signals indicating key husbandry events negatively impact on captive capuchin behaviours.

During the baseline pre-signal data collection, 76 husbandry routines that involved door events were recorded, over twelve days. These included indoor capuchin enclosure cleaning ( $n=14$ ), indoor capuchin enclosure feed ( $n=14$ ), keeper in the squirrel monkey enclosure ( $n=10$ ), and other (indoor maintenance, keeper in the holding area, keeper in testing rooms, keepers in outdoor enclosure, and outdoor scatter feeds) ( $n=38$ ). Only the first two categories involved entry into the indoor capuchin enclosure ( $n=28$ , 37% of all door events), so the sounds of the door openings were unreliable with respect to predicting entry to the indoor enclosure, although cleaning was on a relatively temporally predictable schedule (i.e. occurred every morning before 10am). The monkeys visibly startled when a keeper entered the indoor enclosure. The frequencies of each of the behaviours pre-door events were compared for each type of husbandry routine separately to determine if the monkeys were anticipating the events differently. Scratching and autogrooming, two displacement activities (Maestriperieri et al., 1992) occurred at a higher frequency per individual before keepers entered the squirrel monkey enclosure (4.25/5 min) and the capuchin inside feed (3.79/5 min) than before the indoor clean (1.29/5 min) or other (3.14/5 min) ( $\chi^2(3)=9.50$ ,  $P=0.013$ ). However, these differences were not significant with Wilcoxon tests with Bonferroni correction, and data are collapsed across husbandry routines for further analyses.

As described in the Methods, data were divided into 5 minute segments, before and after a door event and these were compared to determine whether behaviour changed significantly. Three

behaviours were found to be significantly different at the group level: scratching, vigilance and jerky motion occurred at higher frequencies after a door event than before a door event ( $Z=4.4$ ,  $6.4$ , and  $3.0$  respectively,  $P<0.01$  for all).

### **3.2 Hypothesis 2: Making a startling husbandry event more predictable via reliable signalling will decrease anxiety-related behaviours shown in anticipation of the event.**

During the Treatment condition, there were 43 door events of which 30% ( $n=13$ ) involved entry into the indoor capuchin enclosure. The frequency of potentially anxiety-related behaviours in the 5 minutes before and after a door event in both Baseline and Treatment conditions were analysed using Friedman's ANOVA. All four conditions ((A) Baseline before door event, (B) Baseline after door event, (C) Treatment before door event, and (D) Treatment after door event) were analysed against one another to determine significance of individual aspects (see Table 3). Data were collapsed in to four categories ((A+C) Baseline and Treatment before door events, (B+D) Baseline and Treatment after door events, (A+B) Baseline before and after door events, and (C+D) Treatment before and after door events) and analysed using Friedman's ANOVA in order to determine if any main effects were apparent (see Table 4).

Table 3 and 4 about here

Rates of scratching, vigilance, and jerky motions were significantly higher after a door event when compared to before. Scratching and vigilance were also affected by signalling, with lower frequencies after signalling was implemented than before. There was no significant change in jerky motion (see Table 4). Wilcoxon signed rank test *post hoc* analysis, followed by Bonferroni correction, showed the frequency of scratching, vigilance, and jerky motions were all significantly higher in the Baseline stage after a door event, than before (A vs B) with vigilance and jerky motion also being higher in the Treatment stage (C vs D). No significant change was seen in any of the behaviours in the Baseline

to Treatment before door event stage (A vs C). All three behaviours were significantly lower in frequency after the door event in the Treatment stage (B vs D), but, jerky motion was found to be non-significant after Bonferroni correction. Figure 2 illustrates the data which show the signal having a positive impact on these anxiety-related behaviours, with reductions in the Treatment condition after door events.

Figure 2 about here

#### **4. Discussion**

Before deciding if the introduction of a signal should be considered, it is important to assess how behaviour is affected by husbandry routines. Although a very time-intensive method, all day focal samples were the most effective way to collect short duration behavioural responses to external, human-related events that had some temporal unpredictability (e.g. the morning feeding might occur any time between 9-10am). All day sampling allowed for the flexibility of timing of events in a zoo environment without experimentally manipulating the routines.

##### **4.1 Hypothesis 1**

Preliminary analysis was used to determine if there was any justification for signal implementation. Without a clear relationship between the husbandry events and anxiety-related behaviours there is no reason to introduce a reliable signal. The results of analysis suggest that door events significantly increased scratching, vigilance and jerky motions which are all potentially anxiety-related, and potentially undesirable and seen in anticipation of a positive event such as feeding (e.g. Waitt and Buchanan-Smith, 2001). Keeper activity as indicated by door events was shown to have a significantly adverse impact on behaviour. Urine washing, self-grooming, branch shaking and threat faces did not occur at high frequencies, nor in a consistent manner in relation to keeper activity and are therefore not useful as a measure of anxiety in this context, indeed if they are related at all. Previous research has shown that there is a negative impact on welfare by removal of a reliable signal after it has



been implemented (reviewed in Bassett and Buchanan-Smith, 2007). Therefore, before implementation of the signal, we explained to the keepers the need to maintain the signal consistently if it was shown to be beneficial.

The data clearly indicate that sounds of keeper activities through doors openings were impacting on anxiety-related behaviours. All husbandry events were collapsed for analyses, although there was some indication that they were anticipated differently. For example, feeding is an appetitive event, that is likely to be perceived as having a more positive valence than, for example cleaning. The data showed that the lowest frequencies of scratching were before cleaning the capuchin enclosure, and were highest for the capuchin indoor feed and squirrel monkey enclosure entry (which the capuchins could not see but may have been using as an additional signal). This suggests that the animals may have been able to anticipate to some degree the type of husbandry event, based on temporal predictability and sequencing (e.g. cleaning was always in the morning before feeding), and that behaviour in anticipation of events was modified accordingly in relation to perceived valence of the event.

#### **4.2 Hypothesis 2**

The study sought to determine if the addition of a reliable signal before keeper entry into the capuchin enclosures would lower the frequency of anxiety-related behaviours. Results showed a significant decrease in three anxiety-related behaviours. As expected, anxious behaviours were higher after a door event in the Baseline condition than before it, but once the reliable signal was implemented (Treatment condition), this was no longer significant. Behaviours in the before door event period were not significantly different in the Baseline and Treatment conditions, suggesting that the introduction of a signal did not affect behaviours outwith husbandry events, but frequencies were significantly lower in the after door Treatment condition than the after door Baseline condition. This clearly indicates that the addition of a reliable signal, alerting the animals to impending entry by a keeper or other individual, has a positive effect on welfare.

Entry into the capuchin enclosures only comprised 30-37% of door events, primarily in the mornings (every morning for cleaning), but in the Baseline condition, individuals responded to all door events as it indicated something might occur. Entry was previously a sudden event, which the capuchins could not predict, and would often result in startle responses. The introduction of the reliable signal indicating entry by keepers into the internal enclosures appears to have allowed the capuchins to pay less attention to the door openings, and to prepare themselves for keeper entry (as one might prepare when sitting in an office room upon hearing a colleague knocking).

## **5. Conclusion**

Animals have limited ability to control events in their lives in captivity, and are reliant on humans. They use signals present in the environment to predict husbandry events, yet many of these cues are unreliable, leaving the animal in a state of uncertainty. Other events may occur suddenly (such as automatic lights on or off), and surprise or startle the animals. The use of signalling an event is an important tool to inform animals what will happen, and when it will happen. This research has shown that the introduction of a unique reliable signal can reduce anxiety-related behaviours. Such an approach is neither time-consuming nor expensive to implement and therefore should have broad appeal for uptake in a range of captive settings.

## **6. Conflict of interest**

The authors of this manuscript have no conflict of interest that would inappropriately bias this research.

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Figure 1: Schematic diagram of the Living Links to Human Evolution Research Centre (WS/ES = west/east squirrel monkeys, WC/EC = west/east capuchins) with inset showing the lower keeper level with locations of the doors, and the door the keepers knocked on.

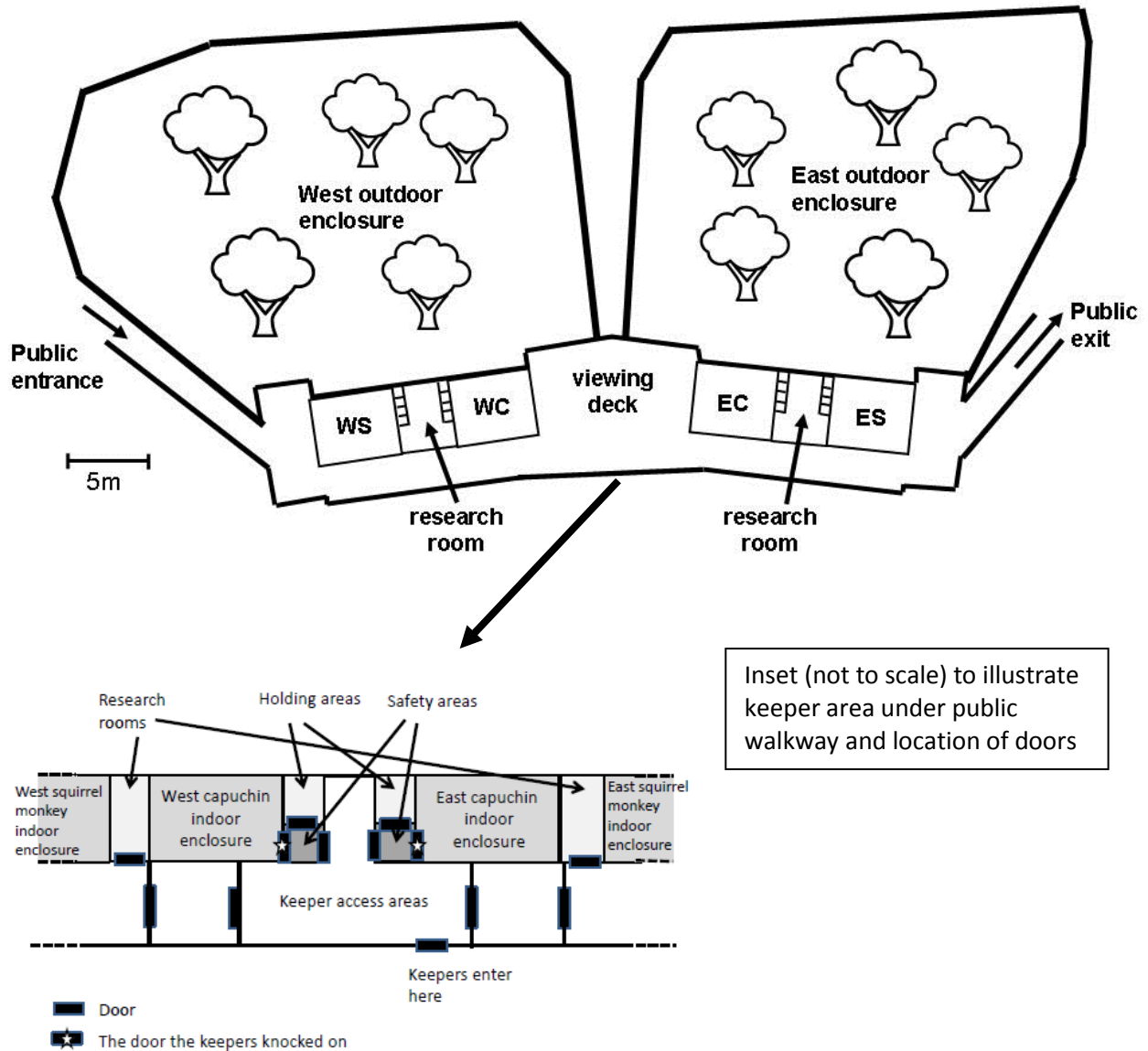


Figure 2a

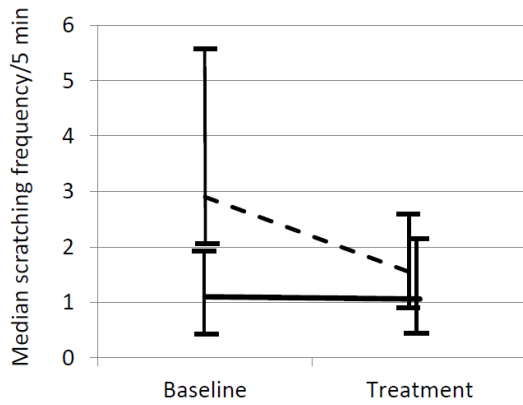


Figure 2b

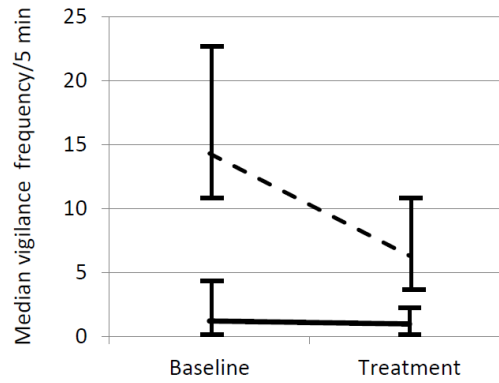


Figure 2c

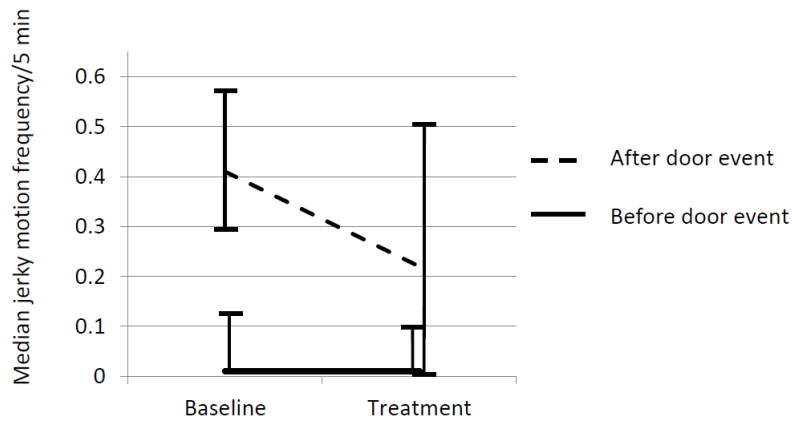


Figure 2: Baseline and Treatment condition comparisons of median (a) scratching frequencies, (b) vigilance (c) jerky motion frequencies per 5 minutes before and after the door events. Error bars indicate 95% confidence intervals.

Table 1: Details (sex, age and rank) of the capuchins studied.

Individual Name (East)	Sex	Age	Rank	Individual Name (West)	Sex	Age	Rank
Anita	F	13	$\alpha$	Lana	F	15	$\alpha$
Penelope	F	5	$\beta$	Santiago	F	8	$\beta$
Junon	F	10	$\gamma$	Sylvania	F	7	$\gamma$
Manuel	M	6	-	Toka	M	6	-
Carlos	M	4	youngster	Figo	M	4	youngster
Chico	M	1	juvenile	Micoe	M	2	juvenile

Table 2: Definitions of behaviours recorded.

Behaviour	Definition
Scratch	Raking of fingers across limbs or body parts, with a new scratching action beginning when a previously scratching hand/foot begins scratching a new body part or has stopped scratching a current body part, and begins scratching the same body part, with a pause in between motions.
Vigilance	Discrete directed gaze with head directed toward a designated 'keeper area' (defined as areas of indoor and outdoor enclosures which could be associated with the presence of a keeper). Each discrete vigilance movement is scored.
Jerky motion	Sudden, quick movement of a body part (including head, neck, torso, and entire body), refocusing attention and gaze. Body jerks are accompanied by fixed gaze toward a human, human-related action or sound.

Table 3: Description of the four conditions analysed.

	Baseline (A+B)	Treatment (C+D)
Before Door Event (A+C)	A	C
After Door Event (B+D)	B	D



Table 4 Results for significance of frequency of scratching, vigilance toward keeper areas, and jerky motions in before and after door event conditions and in the Baseline and Treatment conditions

	<b>Before vs after door</b>	<b>Baseline vs Treatment</b>	<b>A vs B</b>	<b>C vs D</b>	<b>A vs C</b>	<b>B vs D</b>
<b>Scratching</b>	$X^2(1) = 8.3$ P<0.001	$X^2(1) = 5.3$ P=0.039	$X^2(1) = 12.0$ P<0.001	NS	NS	$X^2(1) = 8.3$ P=0.006
<b>Vigilance</b>	$X^2(1) = 12.0$ P<0.001	$X^2(1) = 8.3$ P<0.006	$X^2(1) = 12.0$ P=<0.001	$X^2(1) = 12.0$ P<0.001	NS	$X^2(1) = 12.0$ P<0.001
<b>Jerky Motion</b>	$X^2(1) = 12.0$ P<0.001	NS	$X^2(1) = 12.0$ P<0.001	$X^2(1) = 8.0$ P=0.008	NS	$X^2(1) = 12.0$ P=0.039