Measuring physiological stress in the common marmoset (*Callithrix jacchus*): Validation of a salivary cortisol collection and assay technique

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**Abstract**

Cortisol levels are often used as a physiological measure of the stress response in captive primates, with non-invasive measures of this being an important step in welfare assessment. We report a method of collecting saliva samples voluntarily from unrestrained captive common marmosets (*Callithrix jacchus*), and validate an enzyme-linked immunosorbent assay (ELISA) technique previously unused in this species. Saliva samples were collected from marmosets housed in pairs in a UK laboratory. The assay showed parallelism, precision, accuracy and sensitivity, meeting the criteria typically used to investigate the effectiveness of new analytical techniques. Use of Salimetrics® Oral Swabs considerably increased the amount of cortisol recovered in comparison with previous studies using cotton buds. However, while use of banana on the swabs can encourage chewing, it may in some instances negatively impact on cortisol recovery. Following a likely stressful event (capture for weighing), we also found cortisol levels significantly decreased, possibly due to social buffering or ‘blunting’ of the HPA axis. Order of weighing also had an effect. The method therefore provided an effective non-invasive means of assessing acute changes in cortisol level that may be more useful than previous methods, improving our ability to study physiological aspects of welfare in primates. We discuss methodological considerations, as well as implications of using cortisol as a measure of stress.

1. Introduction

1.1. Cortisol as a measure of stress

When aroused, the body undergoes a set of characteristic changes, including activation of the hypothalamic-pituitary-adrenal (HPA) axis. During activation, the hypothalamus releases CRH (corticotropin releasing hormone), causing the pituitary gland to release ACTH (adrenocorticotropic hormone) into the blood, which in turn causes the adrenal gland to increase the output of glucocorticoids [64], making more energy available for immediate use and preparing the body for increased demands. While HPA axis activation is an adaptive response, very strong or prolonged periods of activation can lead to failure to reproduce [59]; abnormal behaviour [25]; impaired cognitive function [74]; immunosuppression [46], which could increase severity of infections (reviewed in [48]); or heightened risk of cardiovascular and metabolic syndromes (reviewed in [80]), all of which can have substantial implications for the wellbeing of animals.

Cortisol is the main glucocorticoid in many mammals. Numerous studies have therefore used it as an indicator of stress ([47], e.g. *Equus cabalbus*; [52]; *Canis familiaris*; [33]; *Macaca mulatta*; [12,56]; *Callithrix sp.*: [14,50,68]). Baseline samples can be taken, to look at relative stressfulness of certain situations, or a stressor can be imposed to examine HPA axis activation [51]. In this case, the intensity of the response from baseline to post-exposure is thought to reflect the degree of averseness, with large changes in cortisol indicating unusually high activation of the stress response, and so greater psychological and physiological stress [25]. Primates face a number of potentially stressful experiences when kept in laboratories, resulting from the captive environment and routine husbandry procedures, as well as experimental manipulations [5]. Increased cortisol levels have been well documented in primates following stressors such as loud unfamiliar noise and human activity (*Callithrix jacchus*: [14,38]), restraint (*M. mulatta*: [57]), human handling (*Saimiri sciureus*: [34]) and maternal separation (reviewed in [14,51,56]).
C. jacchus: chew voluntarily on collection devices without structured training (e.g. which could not be investigated using metabolites within excreta, and increased significance of a social group member (C. jacchus [38]) have also been shown to be physiologically stressful.

However, the use of cortisol does have its difficulties. Levels vary across the day and season, depend on the history of the individual, the type of stressor, the presence of social companions and the collection method used [14,39,55,56,69]. For example, Johnson et al. [37] provided comprehensive data on blood cortisol levels in C. jacchus, measuring differences depending on sex, social status, housing and time of day, with concentrations ranging more than ten-fold from 31.2 ± 2.8 μg/dl to 317.5 ± 82.2 μg/dl. In the same species, Dettling et al. [21] found that brief separations from the family in the first month of life led to lower basal cortisol levels in 28 day old infants, compared to controls. However, there are no established normal adaptive fluctuations in levels of cortisol [25].

As well as this, there are a number of studies showing decreases in cortisol concentration following potential stressors in common marmosets. For example, Bowell [6] found that salivary cortisol level decreased significantly from baseline levels by 30 min after capture for weighing. Similarly, Cross and Rogers [15] found a consistent decrease in salivary cortisol level in all marmosets after presentation of a snake-model stimulus, although their behaviour indicated this was a clear stressor for them. Why there are such differences in findings is not immediately clear, and demonstrates the complexity of using cortisol as a measure of stress. These studies highlight the importance of collecting contextual and behavioural data to assist with the interpretation of cortisol measurements.

1.2. Collecting and measuring cortisol

Cortisol can be collected from several different mediums, giving researchers options for how to measure the physiological stress response [51]. Blood samples have traditionally been taken, often to determine acute reactions to stressors such as social separation (e.g. [36]). However, this method is often confounded by the stress of restraint or sedation. Urine can instead be collected, which is not invasive compared to controls. However, this method is often confounded by the stress of restraint or isolation stress, although it can be used for the majority of time. All marmosets were socialised to humans from birth, with regular hand-feeding and positive interactions. Marmosets in the colony, in combination with behavioural observations, and the commercial availability of the assay will encourage uptake by other facilities, increasing valid comparisons across studies.

2. Method

2.1. Animals and housing

Twenty-six adult common marmosets, housed in vasedecomposed male mixed-sex pairs in 3 rooms at Dstl, Porton Down, UK were studied (aged between 1 year 7 months and 2 years 7 months). All animals were purpose bred in captivity: 19 were family-reared, 7 received supplementary feeding from caretakers as infants, but remained with the family for the majority of time. All marmosets were socialised to humans from birth, with regular hand-feeding and positive interactions.

Marmosets were housed in cages measuring 100 cm wide × 60 cm deep × 180 cm high, lined with wood chippings and furnished with a nestbox, wooden platforms, perches, ropes, suspended toys and a wire veranda. All marmosets had ad libitum access to water, and food was delivered twice a day. Primate pellets (40/pair) were fed in the morning, and a variety of fruit (1 piece/animal) was provided in the afternoon. This was supplemented with malt loaf, egg, rusk, mealworms, dates, peanuts and bread on alternating days. Gum arabic and milkshake (with added Vitamin D once a week) were also given twice a week, and a constant supply of forage mix was available. Enrichment was introduced once a week, where paper parcels, cardboard boxes or bottles were provided, with forage mixed into sawdust. Temperature and humidity were at 23-24 °C and 55 ± 10% respectively. Lighting
was provided on a 12 h light/dark cycle, with a dawn and dusk phase. Methods were approved after review by the Stirling University Psychology Ethics Committee and the facility involved, and complies with legal and ethical requirements in the UK.

2.2. Study 1: assay validation criteria

Initially, 4 marmosets (2 male, 2 female) provided 5 samples each, using Salimetrics® Oral Swabs (SOS) coated in banana, to assess typical assay validation criteria.

2.2.1. Saliva collection

The monkeys were first habituated to the saliva collection device for 5 min on three days prior to sampling. One end was presented through the wire wall of the home cage, with the other held by the experimenter, and the marmoset allowed to lick and chew the end, depositing saliva onto the swab (following [14]). After approximately 5 min, the collection device was removed and the marmoset given a small piece of banana. All samples were taken between 9:00–10:00.

The collection device was then taken for processing (any containing visible traces of blood, which would affect the cortisol assay, were removed). The device was first cut to approximately 3 cm to fit into the storage tube, and sealed. Samples were marked with subject ID, time and date. The tubes, with their contents, were frozen at −20 °C for less than one week. The samples were then placed into a centrifuge and spun for 15 min at 1500 RPM, to separate the saliva from the collection device. A minimum of 25 μl of saliva is necessary for analysis [61], which was typically collected. The saliva samples were then stored at −80 °C, until being assayed within 6 months. Storage time should not exceed 9 months [1].

2.2.2. Cortisol assay

Samples were analysed using Salimetrics® Salivary Cortisol Enzyme Immunoassay Research Kits. The plate was run as per the manufacturer's instructions [61], using the standards in the range 82.77, 27.59, 9.19, 3.06, 1.02, 0.33 nmol/l. Cross reactivities of the cortisol antibody bodies can be found in Salimetrics [61]. All SOS samples were run in duplicate at a dilution of 1:5000.

2.2.3. Assay validation

The Salimetrics® assay was validated for use in common marmosets, using standard techniques [7]. Serial dilutions of pooled SOS samples, detailed above, were run in conjunction with synthetic standards provided in the kit, to assess specificity. Accuracy was investigated by quantifying the recovery of increasing amounts of synthetic cortisol (0, 9.19, 27.59, 82.77 nmol/l), added to known quantities of sample measured from the pooled saliva (2.43 nmol/l). Coefficients of variation (CV) of low and high concentration quality controls were assessed within and between plates, to identify intra- (N = 3 plates) and inter-assay precision (N = 3 plates). Sensitivity was determined as the smallest concentration of cortisol that could be detected in the working range (the point of 90% B/BO) of the assay [58].

2.3. Study 2: collection method

Six marmosets (3 male, 3 female) provided 4 samples each to assess the collection method (Salimetrics® Oral Swabs vs cotton buds, with and without banana).

2.3.1. Saliva collection

Salimetrics® Oral Swabs are made of a polymer, have verified recoveries of salivary cortisol, and do not cause a change in sample pH. Saliva was collected using the method outlined in Section 2.2.1. Each marmoset was presented with both collection devices (cotton bud first, followed by SOS 5 min later), firstly without banana. Approximately 30 min later, they were then presented with each collection device again (cotton bud first, followed by SOS 5 min later), after rubbing it into a banana for 5 s to coat it with the fruit. This order avoided contamination of the first samples, and has been used previously by Cross et al. [14]. Cortisol was assayed using the above method (see Section 2.2.2).

2.3.2. Statistical analysis

As no transformation was successful in making data normally distributed (assessed using Kolmogorov-Smirnov tests), non-parametric tests were used to assess the saliva collection method. Mann Whitney tests were used to compare cortisol concentration between cotton buds and SOS with and without banana. Spearman’s rank correlations were also conducted, to look at the relationship with and without banana for each collection device. Two-tailed tests were used, with P < 0.05 considered to be statistically significant. All analyses were conducted in SPSS Version 19.

2.4. Study 3: biological validation

Twenty-one marmosets (12 male, 9 female) provided baseline (same time period on normal, undisturbed days in the lab) and post stressor samples on one weighing occasion, to assess biological validity of the assay. All marmosets provided 3 baseline samples each. Eighteen marmosets provided 2 post stressor samples, while the remaining 3 individuals provided only one post stressor sample. In 5 cases, the same animal was sampled in both the biological validation and the collection device studies.

2.4.1. Weighing procedure

Weighing is a necessary routine event, carried out each month, which provides a good opportunity to assess how individuals cope with a mild stressor, without imposing any stress for the sole purpose of the study. Weighing took place between 9:00 and 10:00. The marmoset was caught by grasping the base of the tail and then holding the animal around the chest. After a brief health check, the animal was placed into a small, plastic box and weighed on the scales. They had no visual or olfactory contact with their pair member while in the weighing box, although they were within auditory contact. The box was opened in the new clean cage and the animal allowed to leave at will. The old cage was then removed for washing. The whole process lasted approximately 5 min/marmoset. While in the home cage, the marmosets were in view of other pairs in the room being weighed. Order of weighing (comparing 12 individuals weighed first in the room with 9 individuals weighed last in the room (see [4]) was counter balanced between males and females.

2.4.2. Saliva sampling

Saliva was sampled on three baseline days between 9:00 and 10:00 in the week prior to weighing, with similar timings for each individual animal, to ensure compatibility and avoid variation due to circadian rhythm [13]. Two saliva samples were collected after capture and weighing, at 0–5 min and 25–30 min. Saliva was collected using the method in Section 2.2.1, using SOS with a banana coating, and the assay was conducted as outlined in Section 2.2.2.

2.4.3. Statistical analysis

To look at biologically meaningful changes in cortisol level, means were calculated from the three baseline cortisol values for each individual, to obtain one baseline value for use in the analysis, in attempt to reduce variability. As no transformation was successful in making data normally distributed, Friedman tests were conducted to look at differences in cortisol concentration over the time points (baseline, post 0–5 min and post 25–30 min). Follow-up Wilcoxon tests were conducted to find where the difference lay. Mann Whitney tests were used to look at sex differences at baseline. As data was approximately normally distributed within order of weighing, differences in cortisol...
between those weighed first and last in the room were analysed at baseline (using all 3 values), post 0–5 min and post 25–30 min using Independent samples *t*-tests.

3. Results

3.1. Study 1: assay validation criteria

Displacement curves of serial dilutions of the commercial standards and the pooled saliva samples over the 10–90% binding range did not differ significantly (ANOVA: F (1,16) = 0.944, NS), inferring parallelism between the standards and samples, and so assay specificity. Recovery of the commercial standards (3.06, 1.02, 0.33 nmol/l) added to a low concentration (1:2000 dilution) mixed saliva pool was 101.71% ± 6.26 (r = 0.998, P < 0.0001), and a high concentration (1:1000 dilution) mixed saliva pool was 92.64% ± 5.41 (r = 0.999, P < 0.0001), suggesting good accuracy at both dilutions. Intra-assay coefficients of variation for low and high concentration quality controls were 2.39% and 2.39% respectively (N = 3 plates). Inter-assay coefficients of variation for low and high concentration quality controls were 4.54% and 7.28% respectively (N = 3 plates). Sensitivity, computed from the pooled saliva samples, was 0.86 nmol/l.

3.2. Study 2: collection method

A dilution of 1:1000 was necessary for pooled samples collected by cotton buds to fall within the linear range of the standard curve (i.e. B/B₀ of around 50%), while a 1:5000 dilution was necessary for samples collected by SOS. For cotton bud samples, those without banana had significantly higher cortisol concentrations than those with banana (Mann Whitney tests: U = 0.00, N = 16, P = 0.001). A highly significant positive correlation was also found between cortisol concentrations collected with and without banana (Spearman’s rank correlation: r = 0.98, P < 0.0001). The relationship fit the following equation: without banana = with banana/0.55. However, for SOS samples, those with banana had significantly higher cortisol levels than those without banana (U = 1.00, N = 11, P = 0.011; Fig. 1). SOS samples with and without banana were not significantly correlated (r = 0.70, P = 0.188).

3.3. Study 3: biological validation

In total, 95.06% of samples were successfully collected and analysed. As a banana correction factor for SOS was difficult to identify (see Section 3.2), all data presented were uncorrected for banana. Variation across baseline cortisol measurements was high, ranging from 614.10–28,917.10 nmol/l. Although not significant, females had higher baseline cortisol values than males (mean 9473.34 ± 7833.69 nmol/l v. 6388.47 ± 5530.48 nmol/l).

There was a significant difference in cortisol concentrations across the three time points (X²(2) = 19.86, P < 0.001). Cortisol significantly decreased from baseline to post-capture 0–5 min (Z = −3.82, P < 0.001), and from baseline to post-capture 25–30 min (Z = −3.36, P < 0.001; Fig. 2). Those weighed last in the room had significantly higher cortisol values than those weighed first, both at baseline (t = 2.79, P = 0.007) and at post-capture 25–30 min (t = 2.86, P = 0.013; Fig. 3).

4. Discussion

4.1. Assay validation criteria

The Salimetrics® ELISA performed well on typical tests used to validate an assay in a new species. It was found to have high specificity, demonstrating that cortisol in the samples and standards reacted in a similar manner with the antibody [58], with minimal cross reactivity from other molecules present in the saliva or banana. As the measurement obtained in the assay agreed with the actual amount of the substance when known amounts of cortisol were added to dilutions of the sample, accuracy was also high. Target values of < 5% for intra-assay and < 10% for inter-assay CVs were met [65], and so there was excellent agreement between replicate measures of a known sample, assayed within and between plates. Lastly, as the assay is able to detect even small concentrations of cortisol (computed at 90% B/B₀), sensitivity was high. Comparison of values with a further assay following a chromatographic procedure to purify the cortisol could however confirm validity [10].

4.2. Collection method

Levels of cortisol have been reported in callithricids using saliva [6,14], blood plasma (e.g. [37,77]), urine [69,77], faeces [71,72] and hair [11], with cortisol measurements varying between methods of collection and even between studies using the same collection method (reviewed in [6]). For example, blood plasma concentrations have been reported in adult female *C. jacchus* to range from 182.07 µg/dl [66] to 3858 µg/dl [81].

Mean baseline cortisol level in the present study, using Salimetrics® Oral Swabs, was 7710.56 ± 6735.65 nmol/l. Although not statistically significant, females had approximately one-third higher baseline levels than males, as reported previously in marmosets (*C. jacchus* [37]: blood cortisol; *Callithrix kuhlii* [68]: urinary cortisol), which may be due to the impact of reproductive steroids on HPA axis function [63]. A considerably higher amount of salivary cortisol was therefore recovered in the current study, compared to previously published data. For example, Cross et al. [14] used cotton buds to collect saliva, finding mean concentration at undisturbed baseline periods to be 561 nmol/l. However, this rose to almost 4500 nmol/l in disturbed periods in certain individuals (mean 1198 ± 179 nmol/l). Differences between studies may be due to time of sample collection, with Cross et al. [14] collecting their samples later in the day, at 16:00–17:00, when cortisol has decreased significantly from morning levels. Cross and Rogers [13] found that salivary cortisol in marmosets peaked upon waking (to as high as 1200 nmol/l), then gradually declined throughout the day. They also found high variation in morning samples, during undisturbed periods, which is similar to our baseline findings. Direct comparisons between published studies may therefore not be useful, although relative differences can be found within studies.

Results from the current study showing that a 1:5000 dilution was necessary for SOS, compared to a 1:1000 dilution for cotton buds, suggest that polymer collection devices can recover 5 times more cortisol than cotton collection devices, which is similar to findings by Salimetrics [62] and [29] (Salivette). This finding is likely because SOS are designed for the collection of saliva samples for analysis, being made of a material that filters mucins, cells and other aggregates in the saliva, allowing for greater recovery. Therefore, the use of SOS is recommended over cotton buds.

The vast majority of samples with banana were successfully analysed, and those with no readings were likely because not enough saliva was collected. However, while the relationship between cotton buds with and without banana was comparable to that found by Cross et al. [14] for *C. jacchus* (without banana = with banana / 0.55), as expected due to dilution of the samples with banana, there was unexpectedly no consistent effect of banana on cortisol concentration over collection devices. Although the impact of using sequential presentation of cotton buds then SOS is not known for saliva samples, it is possible that previous exposure to the banana on the cotton bud increased cortisol levels for the subsequent SOS sample, either due to food (humans: [76]) or excitement. To further assess any effect of banana on SOS, recovery of samples with banana could be compared to samples without banana. However, given that banana may confound the data in some way, and that marmosets often chewed on the swabs with no banana, using SOS
without fruit coating is the preferred option.

4.3. Biological validation

Biological validation is necessary to assess whether the assay can accurately reflect biologically meaningful changes in hormone levels in the species [31]. Changes in cortisol concentration were detected following a stressor, with levels significantly decreasing in the marmosets after they had been hand-captured, weighed and placed in a new cage. As habituation to the swabs was carried out, it is unlikely the higher cortisol levels at baseline were due to stress during saliva collection, although may have been related to positive excitement, as, with rare exceptions, the marmosets were always willing to chew on the swabs. Elevated baseline levels could also be due to greater activity (Homo sapiens: [73]), with positive correlations being found between cortisol concentration and levels of locomotion in C. kuhli [69], or because food was more freely available at this time [76]. Behavioural observations would therefore aid in interpretation [4].

While some studies have found significant elevations in salivary cortisol following social isolation and a period of noise and human activity in the animal house [14,38], others have found similar reductions in cortisol post-stressor. For example, all marmosets had a significant decrease in salivary cortisol following presentation of a model snake [15]. This response was unexpected, given the increase in stress related behaviours, including tsik calls, agitated movement and mobbing responses. In a further study, cortisol levels doubled in magnitude when marmosets were isolated from peers in an unfamiliar room, although playback of mobbing (tsik) calls from a familiar conspecific when isolated lead to decreases in cortisol [15]. Increases in these vocalisations were noted following capture for weighing in the current study [4], which may help to explain the decrease in cortisol.

Such stress reduction could be due to social buffering, the ability of a companion to ease the stress of challenging situations [28], resulting in a reduced cortisol peak and faster recovery [51], compared to when facing the situation alone. Much physiological evidence has been found for this, such as Smith et al. [69], who found no change in urinary cortisol levels in Callithrix kuhli after 4 day separations from their group when placed in close proximity to a pair-mate, although cortisol levels rose significantly when they were alone. Alternatively, ‘blunting’ of the HPA axis may have occurred following a prolonged period of stress [44,75], due to increased negative feedback sensitivity to glucocorticoids. In a study of humans, Gallagher et al. [27] found that although unemployed people reported higher levels of stress, they unexpectedly had lower cortisol output than employed people. Such down regulation may be an adaptive mechanism to protect the individual from exposure to high cortisol levels. Overall, these results suggest that decreases in cortisol associated with stress may be a common feature across primates.

Order of weighing in the room also appeared to have an effect on salivary cortisol levels. Cortisol concentration was significantly higher 30 min after capture in marmosets weighed last in the room, compared to marmosets weighed first, perhaps as they had been anticipating capture for longer. Previous research has found a positive relationship between order of blood sampling in a room and plasma cortisol concentrations (M. fascicularis: [22]), suggesting that watching other monkeys undergo routine husbandry or procedures, or lengthy anticipation of a negative event, can be stressful. While this fits the predicted results, it is a little unexpected given the overall decrease in cortisol following weighing. As those weighed last had significantly higher baseline levels than those weighed first (which was not ideal), the result may simply be due to levels returning to these higher baseline concentrations at 30 min post capture. It is possible that as there was no
disturbance 30 min after the last marmosets were weighed, compared to those weighed first (when weighing was still occurring 30 min after their capture), the mobbing calls were then reduced, having less diminishing effect on cortisol levels. However, there was a consistent pattern of results, with both those weighed first and last showing the same decrease in cortisol levels following capture for weighing.

4.4. Methodological considerations

Use of SOS and the commercially available Salimetrics® assay did prove to be a valid way of monitoring salivary cortisol in pair-housed marmosets, confirming this is a promising non-invasive method of measuring acute changes in cortisol- an important tool in animal welfare assessment. However, we do not yet have a full understanding of time course and variation in responses to different stressors in most species of non-human primate [51]. Previous research has found that the salivary cortisol response to an ACTH injection stressor in chimpanzees started to increase from 15 min and peaked at 45 min [30], which is similar to humans. However, New World monkeys have low corticosteroid-binding globulin (CBG) capacity and affinity, leading to exceptionally high levels of cortisol compared to other primates [40], and so salivary cortisol response and half-times in marmosets may be different from other species. Despite this, studies looking at the response to capture and weighing in marmosets have detected significant changes in cortisol concentration from 0 to 30 min post stressor (e.g. [6]). Therefore, 30 min, as used in the current study, should be sufficient to find any changes in cortisol concentration.

Differences in early life history could have also contributed to the range in baseline levels (see [21]). Twins are the usual litter size in wild marmosets, but triplet litters are common in captivity [3], and so intrauterine stress or supplementary feeding of large litters to improve survival may have influenced cortisol reactivity. Other factors could have affected concentrations, such as ovulation in females [63] or undetected blood contamination, which will increase cortisol levels [16].

While validation of a biochemical nature may be beneficial to confirm the validity of the assay, such as ACTH challenge, which is followed by significant elevations of glucocorticoid metabolites [60], purely non-invasive measures were selected in the present study, which also piggybacked on unavoidable, potentially stressful husbandry events. Similarly, plasma matching would require venepuncture, which is likely to be stressful in itself and so influence cortisol levels [56]. Studies do however consistently report correlations between plasma and salivary cortisol levels, both in nonhuman (e.g. M. mulatta: [16]) and human primates (e.g. [8,26]), suggesting that salivary cortisol levels can reliably indicate plasma cortisol levels.

4.5. Using cortisol to assess welfare

Despite potential complexities, there is widespread use of cortisol level as a measure of physiological stress in the captive environment, with HPA axis activity being assessed in a variety of contexts, including management practices, social experiences and abnormal behaviour (e.g. [14,17,57]). However, studies of similar stressors have yielded inconsistent results, with some studies finding reduced HPA axis activity and others finding no differences or increased cortisol levels (e.g. abnormal behaviour: reviewed in [51]), making it difficult to draw firm conclusions about animal welfare. Further, particular conditions which are thought to be inherently stressful have led to lowered cortisol levels, including capture and weighing in the present study, and the HPA axis response to positive stimuli, such as winning a social interaction, can be as large as the response to aversive stimuli, such as social defeat [41]. These results suggest that the magnitude of the response is often simply a reflection of metabolic requirements of behavioural activity [42].

The conventional use of the stress concept does therefore have its...
problems. However, the difference in responses to stressors may be due to the psychological, rather than physical, nature of the situation. For example, increased perception of predictability or controllability, could lead to a decline in the magnitude of the stress response or quicker recovery [42]. It is possible in the current study that by the time the marmosets were back in their home cage, the danger had passed, control had been regained, and the parasympathetic nervous system had dampened the stress response (e.g. [2]). Alternatively, while a passive response is associated with increased activation of the parasympathetic system, resulting in greater fluctuations of cortisol, more active responses involve activation of the sympathetic system, which releases adrenaline [15]. This again highlights the need for contextual and behavioural data.

With accumulating evidence that lower concentrations of cortisol may not always be good and higher concentrations may not always be bad [51], care is needed when using cortisol as an index of wellbeing, particularly when comparing studies using different collection methods. Measuring cortisol may however be a useful addition to other assessments of primate welfare [18], to provide a more holistic insight into their wellbeing.

5. Conclusion

This study demonstrated that Salimetrics® Oral Swabs and Salimetrics® Enzyme Immunoassays are reliable means of recovering salivary cortisol, to assess physiological stress in marmosets. The swabs recovered a much greater range of cortisol than traditionally used cotton buds, improving its measurement. The assay was also validated for use in marmosets, and could be used to monitor acute changes in free cortisol levels, including those associated with capture and brief separation from partners. There is now much empirical data showing decreases in cortisol following a stressor, along with increases in cortisol in response to positive stimuli, challenging traditional views on cortisol as an index of stress. The techniques presented may however aid researchers in deciding the optimal strategy for their work, and when used with other measures such as behavioural observations, could enhance our understanding of primate welfare.

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