The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK

Animal Welfare 2008, 17: 125-138
ISSN 0962-7286

Effect of positive reinforcement training on physiological and behavioural stress responses in the hamadryas baboon (Papio hamadryas)

JK O'Brien*†, S Heffernan‡, PC Thomson† and PD McGreevy†

† Faculty of Veterinary Science, University of Sydney, NSW 2006, Australia
‡ Department of Renal Medicine, Royal Prince Alfred Hospital, NSW 2006, Australia
* Contact for correspondence and requests for reprints: justineo@vetsci.usyd.edu.au

Abstract

*Behavioural and salivary cortisol responses were measured in hamadryas baboons (*Papio hamadryas*) (n = 5) undergoing positive reinforcement training (PRT). Compliance was assessed by collecting behavioural data on desirable and undesirable responses during each training session (33–46 training sessions per male). Saliva was collected before implementation of the training programme (3–4 baseline samples per male) and immediately before and ten minutes after a training session (24–53 saliva samples per male). During training, the incidence of leaving the training area, vocalising and threat displays changed across time. Performance of the desired behaviour (holding a target for increasing increments of time) improved for all males during the study period. Concentrations of salivary cortisol were similar for pre-training and post-training collection times, but both were significantly lower than baseline concentrations. The overall decline in undesirable behaviours and the absence of constantly elevated salivary cortisol suggest that PRT had no adverse effects on animal welfare.*

Keywords: *animal welfare, behaviour, corticosteroid, operant conditioning, primates, salivary cortisol*

Introduction

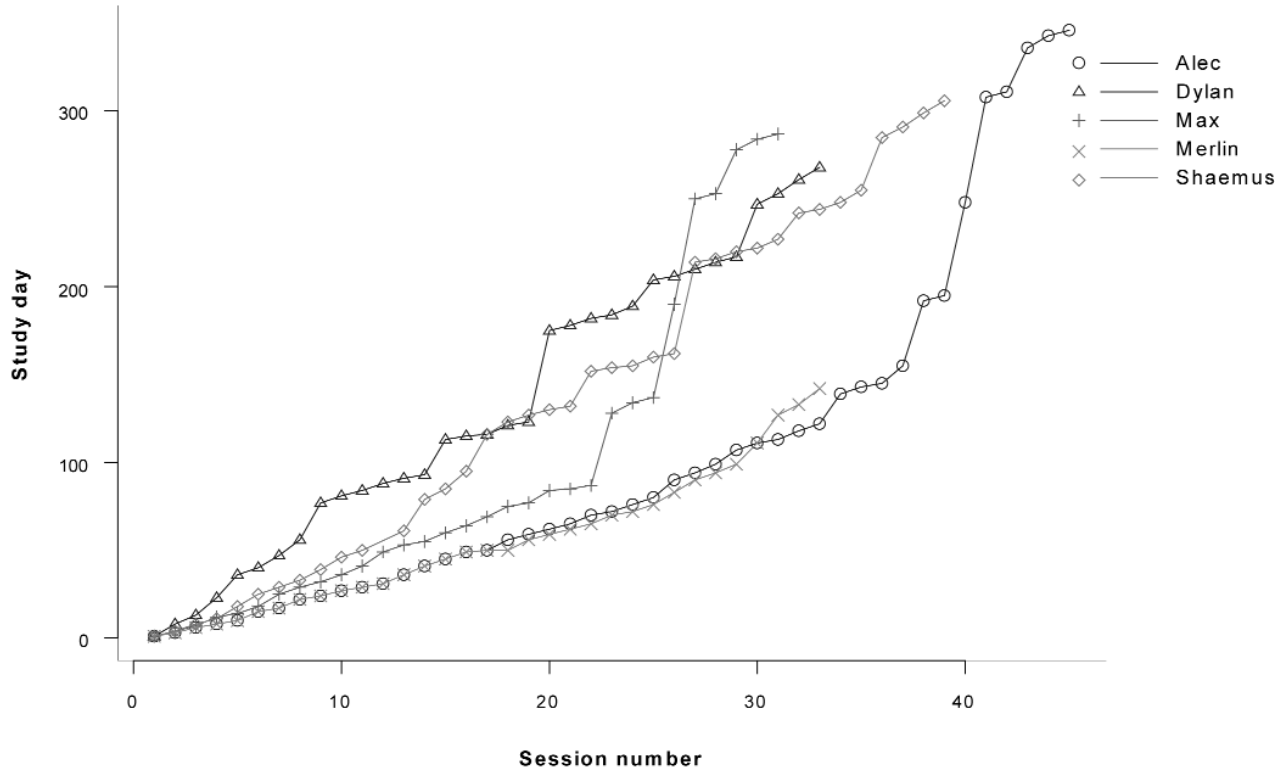
Positive reinforcement training (PRT), a form of operant conditioning, has undergone increased use in zoological institutions since its inception during the 1960s (Breland & Breland 1961). It can enhance animal husbandry and welfare of captive species in numerous ways. For example, training may facilitate husbandry procedures by enabling examinations or biological sample collection without chemical or physical restraint (Desmond & Laule 1994; Laule *et al* 1996, 2003), or even reduce stress-related responses to invasive procedures such as injections (Lambeth *et al* 2006) and thereby improve overall health and reproductive management. Additionally, PRT may provide a form of enrichment for captive wildlife (Kobert 1997; Laule & Desmond 1998; Laule & Whittaker 2007), and assist in managing social behaviour (Bloomsmith *et al* 1994, 1998; Bell & Khan 2001), leading to enhanced captive environments and animal welfare.

Several studies have quantified the impact and effectiveness of PRT through measurement of behavioural responses (Bloomsmith *et al* 1994; Savastano *et al* 2003; Schapiro *et al* 2003). However, only rarely have previous studies systematically measured and reported undesirable responses such as agonistic behaviour during training (Bloomsmith *et al* 1994). Trainers need to monitor agonistic behaviours for the sake of their own safety but also because distress can interfere with learning (McGreevy & Boakes 2007). An understanding of the impact of PRT would be further enhanced by assessment of behavioural and physiological stress parameters in tandem. Responses to stressors involve the release of glucocorticoids (eg cortisol) into the bloodstream via the hypothalamic-pituitary-adrenal (HPA) axis which, in conjunction with accompanying physiological and behavioural responses, enable animals to cope with threatening or demanding situations (Mason 1968; Sapolsky 1990). However, long-term elevation of glucocorticoids is thought to have detrimental consequences for animal learning and memory processes (McEwen & Sapolsky 1995), and may result in suppression of reproductive and immune systems (Munck *et al* 1984; Sapolsky 1990).

Non-invasive assessment of HPA activity, by measurement of circulating corticosteroids, can be performed via saliva sampling (Vining *et al* 1983). Salivary cortisol accurately reflects the biologically-active portion of total plasma cortisol, making it suitable for monitoring physiological stress responses (Kirschbaum & Hellhammer 1989). Saliva has been collected non-invasively for subsequent cortisol assays from several primate species using various methods (rhesus monkeys [*Macaca mulatta*]: Boyce *et al* 1995; Lutz *et al* 2000; squirrel monkeys [*Saimiri sciureus*]: Fuchs *et al* 1997; gorillas [*Gorilla* spp]: Kuhar *et al* 2005; common marmosets [*Callithrix jacchus*]: Cross *et al* 2004). None of these studies reported salivary cortisol concentrations in the context of a training programme.

https://doi.org/10.1017/S0962728600027639 Published online by Cambridge University Press

Figure 1



Time course of training based on chronological time (Study day) and training stage (Session number) for each male.

Positive reinforcement training is commonly used in the captive management of wildlife species, but controlled studies are required to comprehensively evaluate the impact of training on animal behaviour and physiology. The objective of this research was to examine the effect of PRT on salivary cortisol and non-compliant behavioural stress responses in hamadryas baboons (*Papio hamadryas*).

Materials and methods

Procedures described herein were approved by the University of Sydney's Animal Ethics Committee (approval number N02/11-2000/3/3225) and the Central Sydney Area Health Service Animal Welfare Committee (approval number 00/02/AS/02).

Subjects and experimental design

Six adult male hamadryas baboons (Alec, Dylan, Fenton, Max, Merlin, Shaemus), aged 7 years and weighing 18–22 kg were used in the training study. Adult males were selected randomly from a medical research colony population of 130 animals, after satisfying the criterion of not having been involved in previous training. The males were housed together for the duration of the study and 6 months prior to commencement of training. The home enclosure was an outdoor facility (6 × 6 × 4 m; length × width × height) enclosed by steel mesh fences and covered with pebbles. Shade was provided by covering sections of the roof with corrugated metal. The home enclosure also contained wooden logs, trunks and concrete tunnels. The training area (1 × 1.5 × 1 m) was also enclosed by steel mesh and not visible to the home enclosure. Subject Fenton was removed from the study after one month of training, as the volumes of saliva collected from him were insufficient for subsequent cortisol assay. Training of all animals during the same period would have aided in minimising the effect of environmental variation on the data collected. However, the need to collect saliva samples between 0900 and 1100h, to minimise diurnal variation in cortisol secretion, resulted in adequate time for training of, and data collection from, only two animals per day. As such, animals were trained sequentially during the 2.5 year study, with at least two sessions per week for the first 1.5 months of training and at least one session a week thereafter, for a period of 6 to 11.5 months per male (Figure 1).

Operant conditioning, employing a clicker to provide secondary reinforcement (clicker training; eg Pryor 1975), was used to shape the desired response. Incidentally, the training programme was implemented to investigate the feasibility of non-invasive semen collection in the hamadyras baboon and ensure operator safety during training sessions. As described previously in gorillas (Brown & Loskutoff 1998), baboons were trained to sit quietly in a 'safe position'

https://doi.org/10.1017/S0962728600027639 Published online by Cambridge University Press

with their hands on targets placed above head height. The desired response for the baboons was to sit in the training area with their hands on two targets (10 cm of plastic tubing with a diameter of 2.5 cm) for at least 10 s (Figure 2). The targets were raised incrementally during the training from a height of 37 cm above the cage floor to reach a final position of 78.5 cm from the cage floor. The bridge between the behaviour and the reinforcer was an audible 'click' from a hand-held clicker device. Initially, the activation of this mechanism occurred immediately upon performance of the correct behaviour (touching the target) to indicate the imminent arrival of a primary reinforcer to the animal, thus terminating the behaviour. The primary reinforcer was a dried fruit mix (Sunbeam, Victoria, Australia) supplemented with various sugar-based treats (Allen's Confectionery, Nestlé, Australia). These food reinforcers were provided through the mesh of the training area on a continuous reinforcement schedule, although the criteria for reward were made stricter as the animal's behaviour was progressively shaped (McGreevy & Boakes 2007). Each baboon showed evidence of shaping by increasing the duration of holding the targets.

Training sessions were conducted only if the animal voluntarily entered the training area from his home enclosure (this occurred for 97% of the planned training sessions). The trainers (one male and one female) were familiar to the baboons and wore green smocks to herald to the animals the commencement of training (as distinct from a medical procedure, for which blue smocks were worn). In addition, animals were trained in an area in which no adverse procedures had been conducted. Although efforts were made to standardise the duration of each training session, daily variations occurred as a reflection of the motivation and consequent participation of each male (training session length across all baboons: 7.2 ± 3.0 min). Both trainers participating in the study trained each animal but for different numbers of sessions, a variable that was included in subsequent analysis.

Behavioural data collection

All responses by baboons were recorded by the trainers for the duration of each session. In addition, training sessions were also videotaped for later analysis. Undesirable responses were all those that ran counter to the shaping process and thus were of particular interest as an inverted indicator of training success. These responses included agonistic responses towards the trainer and social behaviours, such as vocalisation, that were incompatible with training and took the baboon (or its attention) away from the trainer. So, these behavioural data collected included: (1) the number of times the animal left the training area (since a typical first response from an animal confronted with a stressor is to undergo a flight response ie attempt to withdraw [Moberg 2000]), (2) the number of vocalisations, including screams and barks (Kummer 1968; Estes 1991) and (3) the number of threat displays (Kummer 1968; Estes 1991) including raised eyelids (raising of the eyelids and eyebrows by 'retracting the scalp' and thus displaying the contrastingly pigmented eyelids), yawning (wide-mouthed displaying of the dental arcades), mock approaches (sudden, threatening movements towards the front of the training area), slaps (hitting of the mesh barrier) and biting of the cage structure.

Figure 2

Photograph of a hamadryas baboon holding two plastic targets attached to the mesh of the training area.

Saliva collection and handling

For each male, three or four saliva samples were collected prior to the commencement of the training programme to provide baseline salivary cortisol concentrations. These baseline samples were collected after males had been housed together for at least 2.5 months. Once training commenced, saliva samples were taken both immediately prior to the training session and 10 min after its completion. Pre- and post-training saliva collection was performed in initial sessions (first month of training) and in every second or third training session thereafter. Initially, saliva collection was attempted by enticing the baboon to lick a cotton wool applicator tip, (Jumbo cotton applicator, 180 mm, Livingstone International, NSW, Australia). However, following centrifugation (1,000 g, 15 min) of the cotton wool applicator tip in a 10 ml polypropylene tube (Sarstedt, Australia Pty Ltd, SA, Australia), saliva volume was very low (< 10 ml). A saliva collection device modified from that developed for macaques (Lutz *et al* 2000) was successfully used. The device was made of two perspex plates (Perspex, a transparent acrylic resin, was 65 mm thick, top plate measured 130 × 150 mm [length × width], bottom plate 85 × 110 mm) screwed together. The bottom plate was solid and complete, whilst the top had a central panel (measuring 60 × 80 mm) missing from it. A plastic backboard, a gauze pad and fibreglass mesh (12 × 14 mm with 1.5 mm squares) were placed between the two plates. Raw sugar crystals (CSR, Milton, QLD, Australia) were lightly sprinkled on top of the mesh to encourage the animals to lick the device. The gauze pad absorbed the saliva,

https://doi.org/10.1017/S0962728600027639 Published online by Cambridge University Press

while the mesh prevented significant amounts of sugar being incorporated into the gauze.

The number of times the animals licked the gauze collection device was standardised (between 20 and 25 licks). This minimised the variation in the volume of saliva collected before the sugar residue became less attractive to the animals. Despite this, there were some samples with insufficient volume for analysis (n = 17) that were removed from the data set. Immediately after saliva collection, the gauze swab was placed in a 10 ml polypropylene tube containing a 20–200 ml pipette tip (Sarstedt) and stored at 4°C for no longer than 2 hours. Tubes were then centrifuged (1,000 g, 15 minutes) and the resultant saliva sample (positioned at the bottom of the tube) transferred to a cryovial for storage at –80°C. Previous studies performed in our laboratory using human saliva demonstrated no effect of the cotton gauze swab or sugar crystals on salivary cortisol concentration (AJ Peel/JK O'Brien personal observation 2001).

Saliva analysis

Cortisol concentrations in a total of 163 baboon saliva samples were determined using a ^{125}I cortisol RIA kit ('COAT-A-COUNT', Diagnostic Products Corp, California, USA). Aliquots (25 ml) of samples, standards (0, 1, 5, 10, 20 and 50 mg dl^{-1}) and internal controls were incubated with 1 ml of ^{125}I cortisol at 37°C for 45 min, decanted, and counted for 1 min in a gamma counter (Wallace Oy, Finland). Standard curves were produced by ASSAYZAP (BIOSOFT, Ferguson, MO, USA), and counts were converted into concentrations (nmol l^{-1}). Mean cortisol concentrations were reported as geometric means (derived from exponentiation of the arithmetic means of the log-cortisol data).

Before the training programme commenced, 11 additional adult male baboons were used as the subjects of a pilot study to determine the relationship between salivary cortisol and serum cortisol concentrations. Males were sedated with ketamine (5–8 mg kg^{-1}) for collection of saliva and blood using a cotton gauze and venipuncture, respectively, within 15 min of sedation. Samples were frozen and stored at –80°C until analysis.

Statistical analysis

Behavioural variables involving observational counts (reinforcers [clicks], agonistic behaviours: subject leaving the training area, vocalisations, threat displays) were analysed using generalised additive models (Hastie & Tibshirani 1990). These are an extension of generalised linear models, which can be used for modelling non-normal data such as behavioural counts, but they also allow the mean count to be modelled flexibly with time by use of smoothing splines without imposing a particular functional form, and catering for individual animal responses. The model was specified as:

$$\log\mu_{ijt} = \text{constant} + \text{animal}_i + \text{trainer}_j + s(t)$$

where μ_{ijt} is the mean behavioural observation count; constant is the overall log-mean of the behavioural observation count; animal_i is the effect for the i^{th} animal, being the difference between the log-mean for animal *i* and the overall log-mean; trainer_j is the effect of the j^{th} trainer, being the difference between the log-mean for trainer_j and the overall log-mean; and $s(t)$ is a smoothing spline function of time (either session number or study day). In some situations separate spline functions were fitted for each animal, $s_i(t)$, indicating separate trends for each animal (ie animal × time interaction). A logarithmic link function and Poisson error structure allowing for over-dispersion were specified. Significance tests were conducted using deviance difference and Wald χ^2 tests, and threshold significance was set at $P = 0.05$. Modelling was undertaken using S-PLUS (Insightful Corporation 2001).

Hold duration, as well as the cortisol variables were log-transformed prior to analysis. This was done because of the positively-skewed distributions, and the unstable variances associated with both these measures. However, they were subsequently modelled in a similar way to the behavioural count data, except that a normal error structure was specified. Results were reported on a back-transformed scale, ie geometric means. To assess associations between the various cortisol and behaviour measures, correlations were obtained between the cortisol measure for each animal and the estimated 'animal' effect from the relevant behaviour model, as described above. However, it should be noted that since each correlation is based on only five observations, these tests have little power. Correlations were also obtained between various cortisol measurements (eg salivary and serum cortisol concentration), and simple linear regression was also used to quantity these associations. The analyses were undertaken using S-PLUS (Insightful Corporation 2001).

Results

Behavioural responses

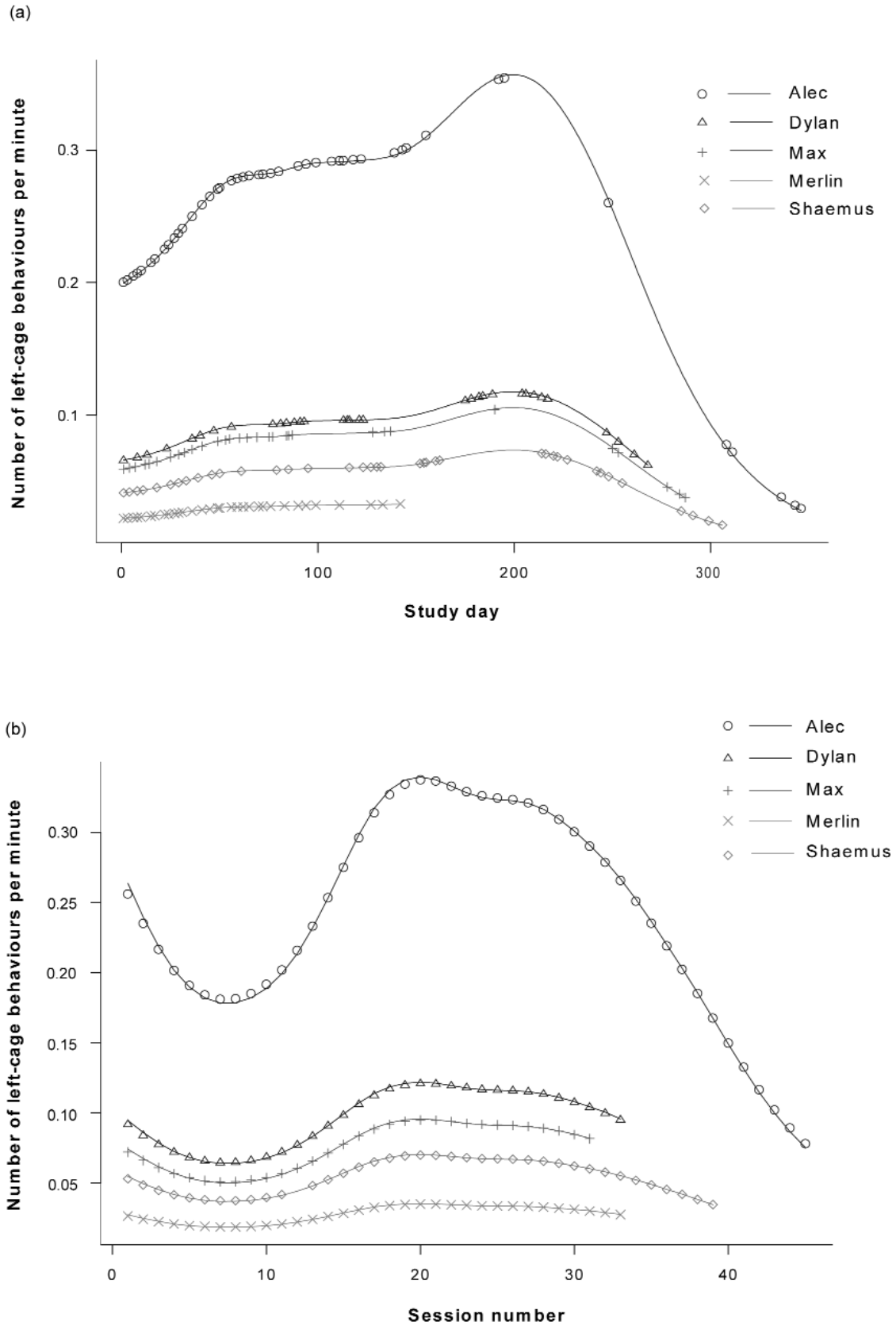
Although it is widely accepted that training effects such as response rates, delivery of rewards and latency to reach criteria are related to the number of training sessions, with little or no effect of time between sessions (eg Thorndike 1898), training was monitored on both chronological and session number scales to provide a species- and context-specific perspective. Accordingly, data are presented as behavioural responses across chronological time (study day) and training session number.

Departures from training area

The number of departures per training session was low (0.8 ± 1.5, data pooled across males and time). There was a significant change in the frequency of departure from the training cage per minute of training across time (based on study day [$P = 0.004$] and session number [$P < 0.001$]) (Figure 3). Based on time-course profiles, there was some evidence for an initial reduction in the frequency of leaving the cage (up to the tenth training session), followed by an increase up to around the twentieth session (about day 200), with a subsequent decline. The frequency of departures from the training area per minute of training was significantly influenced by subject, with Alec displaying this behaviour more often than other males (all $P < 0.001$).

https://doi.org/10.1017/S0962728600027639 Published online by Cambridge University Press

Figure 3



Frequency of leaving the training cage. Horizontal axes represent (a) Study day and (b) Session number. The curves plotted are the GAM smoothers for each animal, with separate time models for both timescales.

https://doi.org/10.1017/S0962728600027639 Published online by Cambridge University Press

Vocalisations

The number of vocalisations during training was low (0.52 ± 0.51 vocalisations per session, data pooled across males and time). The frequency of vocalisations per minute of training was influenced by subject ($P < 0.001$), trainer ($P = 0.013$) and time (based on study day [$P < 0.001$] and session number [$P < 0.001$]) (Figure 4). There was a significant subject × time interaction ($P < 0.001$), reflecting the different time course profiles for each subject. One subject (Max) did not vocalise during any training session.

Threat displays

The number of threat displays during training was 1.30 ± 0.57 displays per session (data pooled across males and time). The frequency of threat displays per minute of training was influenced by subject ($P < 0.001$) and time (based on study day [$P < 0.001$] and session number [$P < 0.001$]). Time course profiles shown in Figure 5 indicate that an increase in the frequency of threat displays occurred during the beginning of the training programme. On chronological and session number time scales, there was a steady decline in the number of threat displays to approximately study day 100 and session 10, respectively. Thereafter, the frequency of this behaviour was relatively constant.

Rate of learning: length of target holds

Each animal increased the period over which they sustained the desired behaviour (sitting whilst holding the targets) over the course of their training ($P < 0.001$). On a log-time scale (Figure 6), there was a significant animal effect on mean hold duration ($P < 0.001$). Specifically, Max demonstrated an increased rate of learning compared with Dylan, Merlin and Shaemus (all $P < 0.05$), and a tendency for increased rate of learning compared with Alec ($P = 0.07$). While the rate of learning by Merlin was similar (both $P > 0.10$) to Dylan and Alec, and higher than Shaemus ($P = 0.036$), Merlin was the only male who did not reach the criterion of consistently holding targets for at least 10 s.

Across all subjects, the rate of learning observed initially began to slow after approximately day 200, corresponding to a coincidental reduction in training session frequency (Figure 7). However, when plotted against the training session, the rate of learning appeared to be sustained (with the exception of Merlin). Figure 7 describes an exponential increase in mean hold time of targets during training and suggests that the interval between training sessions (beyond day 200 or session number 26) was not a limiting factor in learning rate. On a session number timescale, there was a significant animal × time interaction ($P = 0.020$), with this effect being mainly attributed to Merlin. Such interaction represent instances in which additional training sessions would be needed to overcome an observed learning hiatus.

Frequency of reinforcers

As the hold durations improved over time, the number of reinforcers decreased ($P < 0.001$; Figure 8). This inverse relationship was a product of the time limit for each session with longer holds occurring less frequently than shorter holds as the latter were performed more often within each finite training period. Consequently, the clicker sounded less often when behaviours were being sustained for longer. In addition to time, there were significant effects of animal and an animal × time interaction (both $P < 0.001$). Of note is the profile for Shaemus, illustrating that he received a higher rate of reinforcement than other males. This reflects individual variation in response to PRT and subsequent variation in the process of shaping the baboons' ability to perform the desired behaviour.

Salivary cortisol

The validation study using samples from 11 adult male baboons demonstrated significant correlation of baboon serum and salivary cortisol ($r = 0.884$, $P < 0.001$). Serial dilutions (1:1, 1:2, 1:4) of two serum cortisol samples yielded displacement curves parallel to a normal standard curve. Parallel dilutions were not conducted using saliva samples due to the low concentration of cortisol.

Numbers of pre-training and post-training saliva samples submitted for cortisol analysis were: Alec, 27 and 26; Dylan, 15 and 14; Max, 11 and 13; Merlin, 19 and 18; Shaemus, 15 and 12, respectively. Mean cortisol concentrations, reported as geometric means, were similar ($P = 0.3$) for baseline, pre-training and post-training collection times but both of these were significantly lower than baseline concentrations ($P < 0.001$) (Table 1). There was a tendency for cortisol concentration to differ across males, but this was not significant ($P = 0.09$). Cortisol concentration at baseline was not correlated to pre-training, post-training or average cortisol (all $P > 0.05$), although the power to detect a correlation is poor, being based on the five animal averages. However, there was a significant correlation between pre- and post-treatment log-cortisol concentrations (using all paired pre- and post-training data, $r = 0.763$, $P < 0.001$), the least squares regression fit being:

$$\text{logPostCort} = 0.575 + 0.7985 \times \text{logPreCort}$$

Relationship between salivary cortisol and behaviour

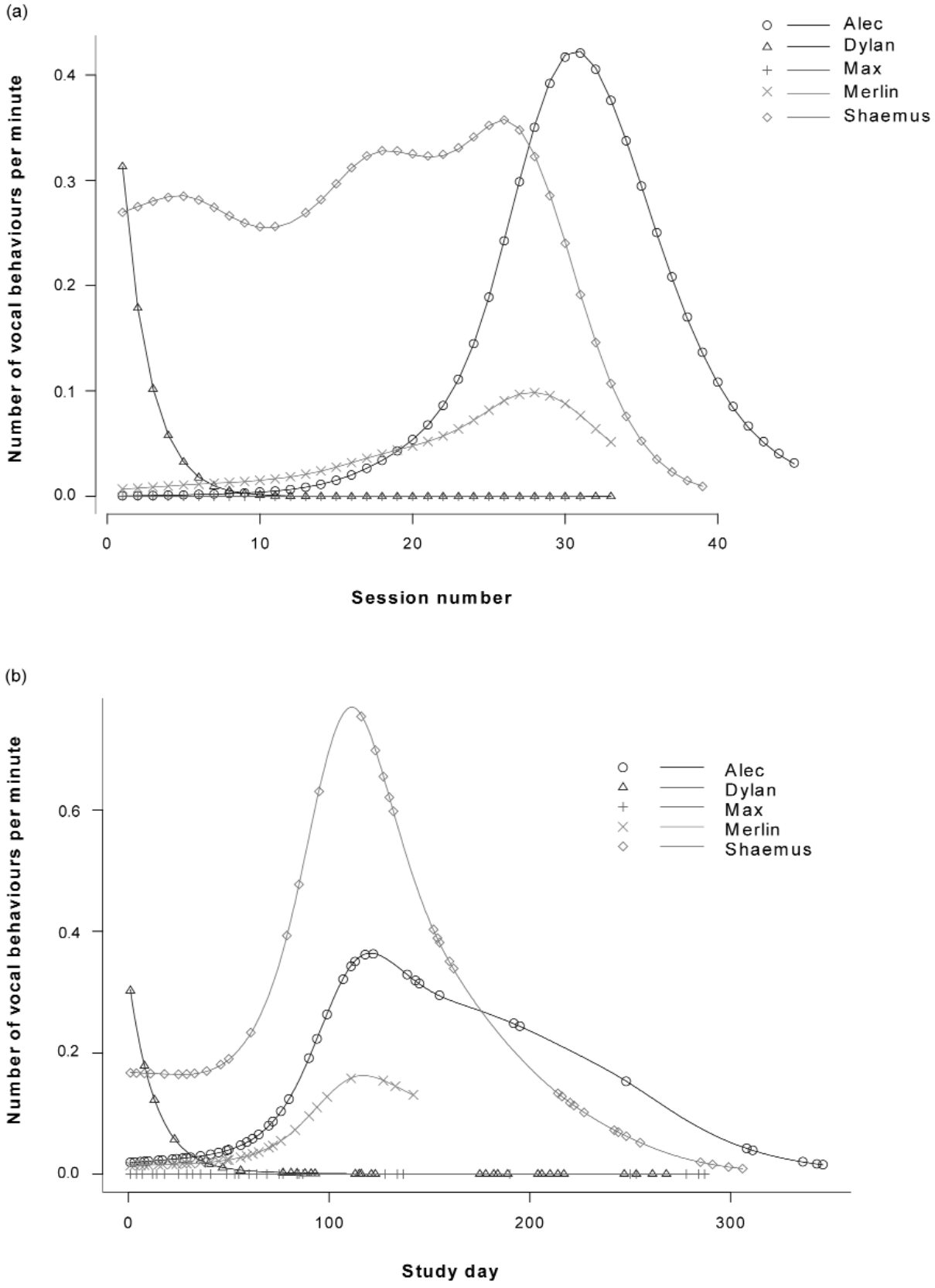
Correlation analyses demonstrated that cortisol concentration was associated with variation in frequency of departures from the training area, with higher cortisol concentrations being associated with greater frequencies of departures ($r > 0.95$, $P < 0.05$, specific values depending on which log-cortisol measure [pre-training, post-training, or average] and animal-specific departure measure [using day or session number] is analysed; Figure 9). However, cortisol concentration could not account for variation in frequency of threat displays ($P > 0.4$, depending on the correlation measure). Similar analyses between cortisol and frequency of vocalisations could not be performed due to animal × time interactions ($P < 0.05$) implying that the correlation would be changing over time.

Discussion

The absence of distress in trained animals is implied by their participation in the training sessions (Hemsworth & Barnett 2000; Moberg 2000). The baboons in the current

https://doi.org/10.1017/S0962728600027639 Published online by Cambridge University Press

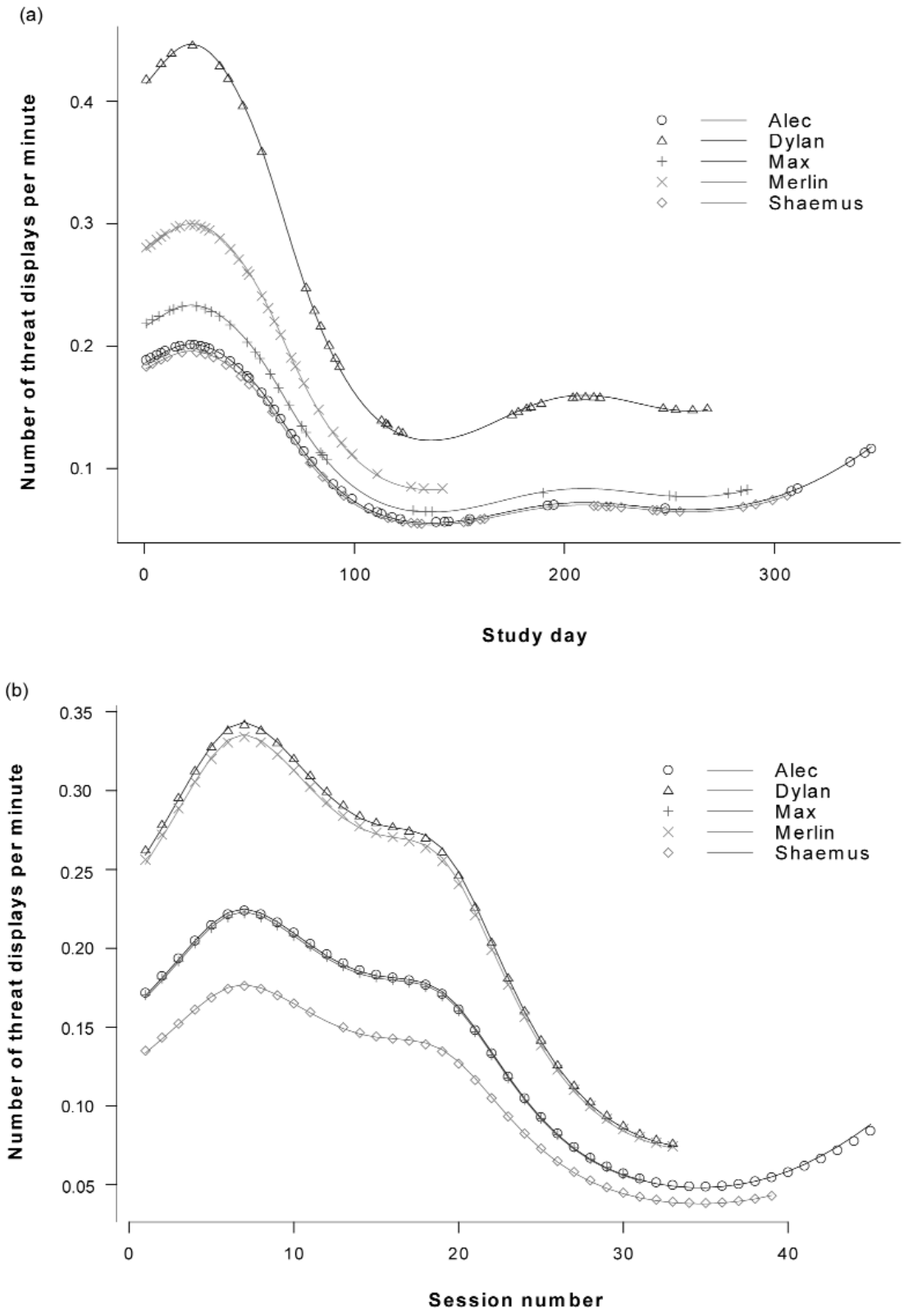
Figure 4



Frequency of vocalisations. Horizontal axes represent (a) Study day and (b) Session number. The curves plotted are the GAM smoothers for each animal, with separate time models for both timescales.

https://doi.org/10.1017/S0962728600027639 Published online by Cambridge University Press

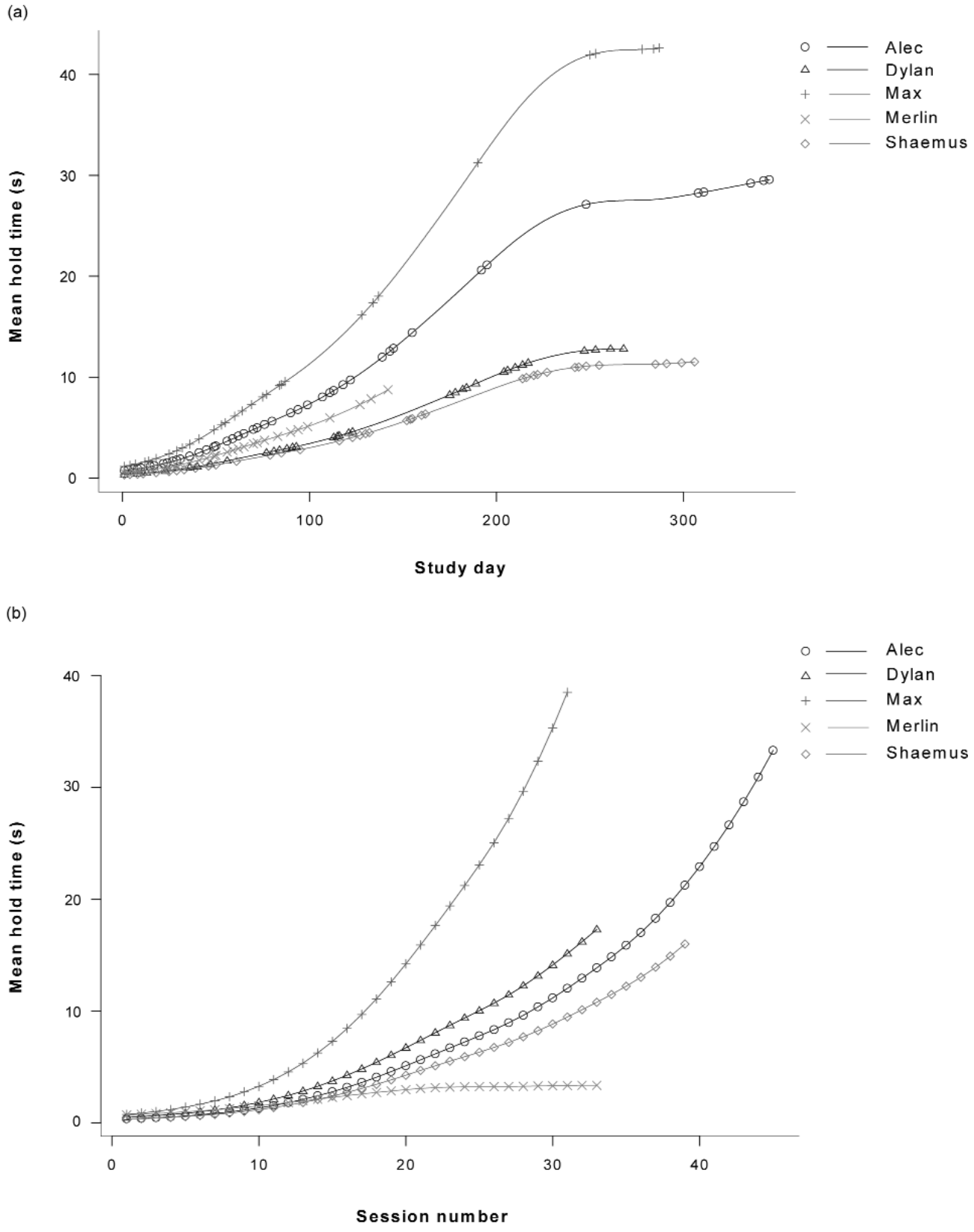
Figure 5



Frequency of threat displays. Horizontal axes represent (a) Study day and (b) Session number. The curves plotted are the GAM smoothers for each animal, with separate time models for both timescales.

https://doi.org/10.1017/S0962728600027639 Published online by Cambridge University Press

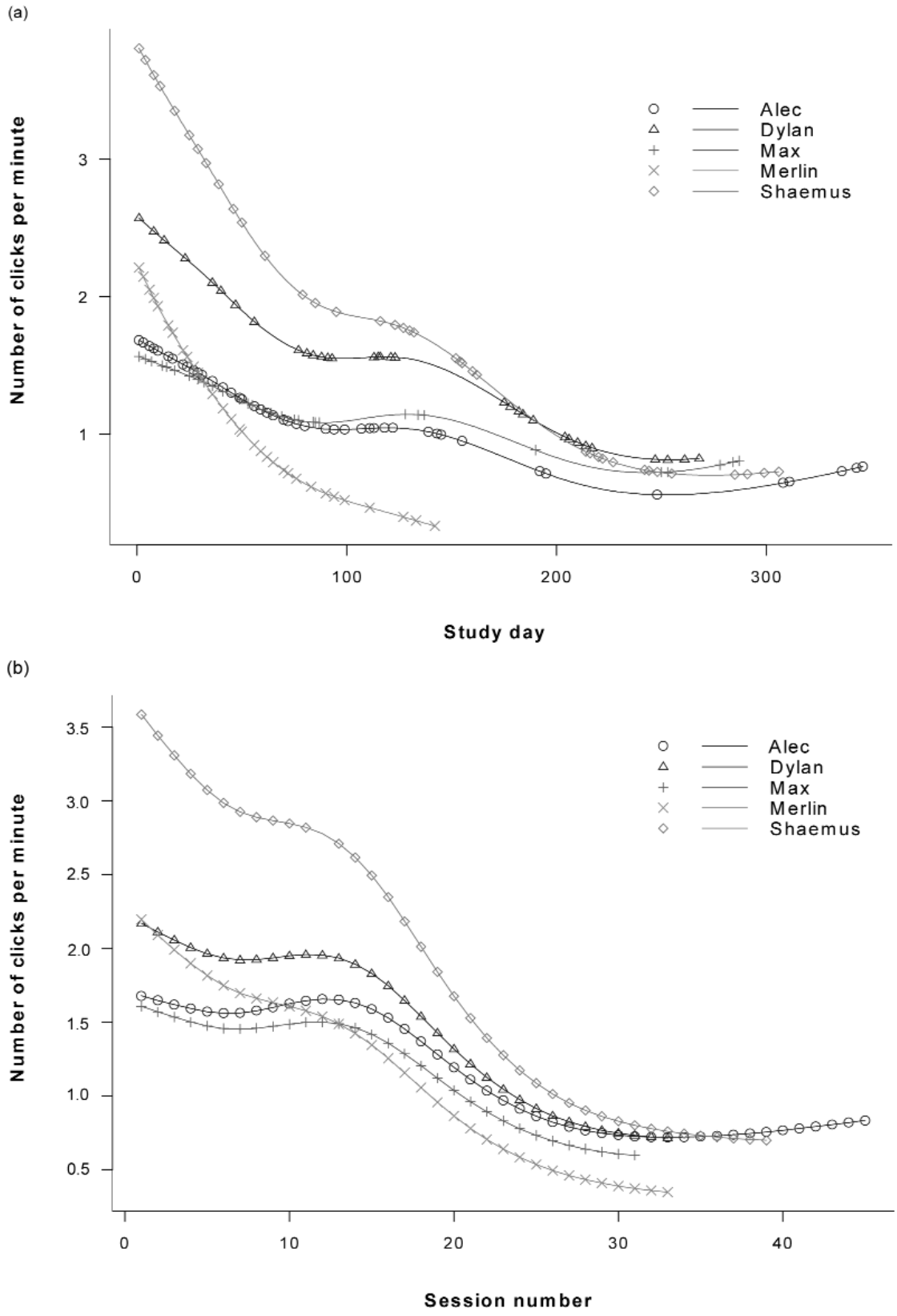
Figure 6



Mean hold time of targets during training. The horizontal axes represent (a) Study day and (b) Session number. The curves plotted are the GAM smoothers for each animal, with separate time models used for both timescales.

https://doi.org/10.1017/S0962728600027639 Published online by Cambridge University Press

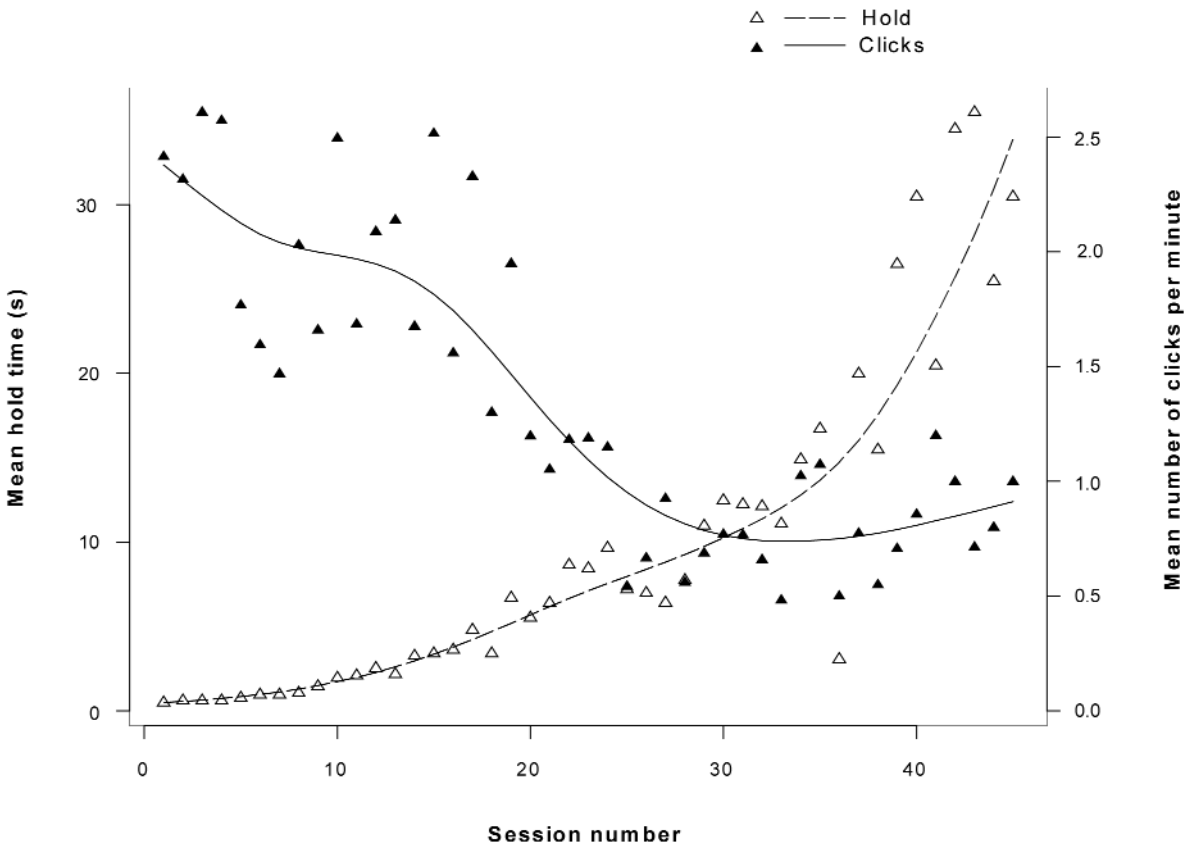
Figure 7



Frequency reinforcers (clicks per minute) during training. The horizontal axes represent (a) Study day and (b) Session number. The curves plotted are the GAM smoothers for each animal, with separate time models for both timescales.

https://doi.org/10.1017/S0962728600027639 Published online by Cambridge University Press

Figure 8



Scatterplot showing the inverse relationship between frequency of reinforcers (clicks per minute) and length of the desired behaviour (holding targets). The symbols represent geometric means taken over all five animals. The superimposed curves are derived from the GAM smoother, to show overall trends.

Table 1 Salivary cortisol concentration statistics (nmol l^{-1}) prior to commencement of training programme (Baseline), immediately prior to training (Pre-training) and 10 minutes following training (Post-training). Data pooled across five males.

	Baseline	Pre-training	Post-training
Number of saliva samples	19	87	83
Geometric mean	43.9	24.4	22.7
SD (log_e cortisol)	0.82	0.59	0.61
Minimum	8	9	7
Maximum	126	106	95

study were not food-deprived and their participation was voluntary as they entered the training area without coercion and could leave at any time. Concurrent analyses of behaviour and salivary cortisol in the present study demonstrated that PRT as a means of modifying behaviour did not adversely affect animal well-being.

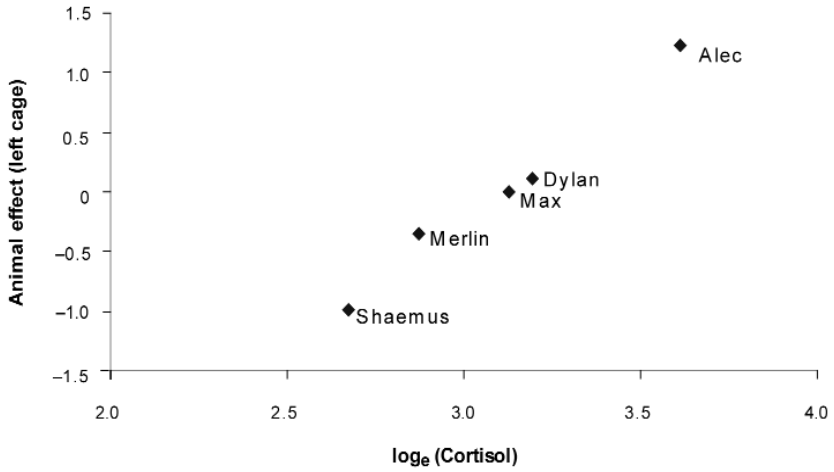
As mentioned previously, one of the six original subjects was removed from the study due to insufficient saliva volumes for cortisol analyses. While it is possible that alternative attractants or absorbent material could increase the saliva volume collected from this individual, additional trials would be required to confirm such components do not affect measurement of cortisol concentration by the radioimmunoassay (Morelius *et al* 2006).

Each animal in the present study showed a reduction in at least one unwelcome response; suggesting that, over time, the training and context-specific stimuli associated with it were all progressively less threatening. The absence of any change in salivary cortisol concentrations over the months of training supports this view. Taken together,

https://doi.org/10.1017/S0962728600027639 Published online by Cambridge University Press

Figure 9

Plot of the log (average cortisol) versus the estimated animal effect for 'left cage' based on chronological time ('study day' model); $r = 0.002$, $P < 0.001$.



these results indicate that any potential negative effect of training on the well-being of the animals was outweighed by benefits, including positive reinforcements (in the form of food rewards), or obviated by habituation. Nevertheless, possible species-specific disadvantages of behavioural modification in certain captive settings should not be overlooked. Separation from the home group may compromise social stability and training may jeopardise appropriate fear of humans in animals intended for release to the wild (McGreevy & Boakes 2007).

Along with timing, consistency is the key to effective training and this study has demonstrated that trainers can be consistent over time and that differences between them can be negligible. This conclusion is supported by the absence of any effect of trainer on the rate of learning. This suggests that, with adequate operator training, compliance and monitoring, trainers can be sufficiently consistent to replace one another. In contrast, the individual variation in responses among the subjects in the current study was striking. When hold duration and number of clicks were considered as a function of study day or session number and the mean hold duration and mean number of clicks were combined for all males, the shape of the profiles differed significantly between animals, as confirmed by the significant time by animal interactions (both study day and session number).

As one might predict, given that hold duration steadily increased, the number of clicks per minute declined consistently for all animals. When one examines the number of clicks (and hence rewards) per minute, it is worth considering the extent to which animals were able to exploit the training opportunity. Animals that required shorter intervals between reinforcements to be shaped to a given threshold , (such as Shaemus), may have derived more direct benefits than those that learned quickly (such as Max). All practical trainers will recognise the dilemma here. The use of too lean a reinforcement schedule can increase the risk of the animal losing interest in the conditioned activity. On the other hand, if trainers relax the criteria and increase click frequency to reignite an animal's engagement in a shaping activity, it is possible that they may inadvertently train it to perform poorly. Certainly, there is an ongoing debate about the need to provide a primary reinforcer after every secondary reinforcer (Rubin *et al* 1980; Cooper 1998; McGreevy & Boakes 2007).

Potential disadvantages of compliance may have existed for baboons in the current study. Holding the handles and assuming the posture necessary to do so, may have been tiring and may have increased vulnerability, especially when in close contact with a human, however familiar. Time away from the home group may have been associated with the risk of social flux and with the perceived loss of opportunities to engage in strategically-critical group activities, including socialisation and foraging. The extent to which these influences may have changed the learning curve for Merlin, when compared with the other baboons, is unclear. However, this study highlights the merits of monitoring progress in individual animals during training to increase efficiency of training and thereby ensure that trainer time is well spent.

Measurements over time (such as number of agonistic behaviours, hold durations and cortisol concentrations) should be assessed using both study day and session number to establish whether responses are changing steadily over chronological time or with the actual session. For example, the plot of study day showed smaller increments in hold duration after approximately day 200, corresponding to a reduction in the frequency of training sessions. However, in the plot of training session, the rate of increase appeared

https://doi.org/10.1017/S0962728600027639 Published online by Cambridge University Press

sustained (with the exception of Merlin), as indicated by an approximately exponential increase in mean hold duration. As observed in other species (Rubin *et al* 1980), this confirms an accumulative approach to learning in hamadryas baboons that renders intervals between training sessions secondary in importance to the quality of the training sessions themselves. Differences in individual animals' apparent ability to retain salient associations (ie remember) have been emphasised by the current data. For selected animals, this may help to justify breaks in training programmes that align with interruptions in staff availability.

Mean cortisol concentrations were similar for pre-training and post-training collection times but both of these were significantly lower than baseline concentrations. The relatively high cortisol concentrations of baseline samplings compared with those observed during PRT most probably reflect prior associations with medical procedures in the collection area and the declining novelty of the saliva collection apparatus. Despite the expected variability in salivary cortisol profiles among subjects, a significant correlation between pre- and post-treatment log-cortisol concentrations was demonstrated. Since there were no signs of any systematic differential in pre- versus post-training cortisol concentrations, the two could effectively be combined via the geometric mean. Analysing these means on a log-scale revealed no significant effect of time (with the exception of the difference between baseline and training sample cortisol concentrations), but an indication of possible between-animal differences. Together, these findings indicate that the animals were either not distressed by training or that they never habituated to it. In the light of concurrent behavioural data, the former seems the most plausible. Basal cortisol concentrations did not correlate with pre- or post-training (nor average) concentrations. So, predictions of ability to cope with stressors in the future should not be made with baseline samples.

A correlation between cortisol concentration and frequency of departures from the training area was detected in the present study. Specifically, higher cortisol concentrations were associated with greater frequencies of departures during training, but cortisol profiles over time did not change for all males. Interestingly, cortisol concentrations did not reflect variation in frequency of threat displays nor vocalisations.

Agonistic behaviour, leaving the cage and vocalisation patterns differed with time and between animals, suggesting that these three separate behavioural categories should not be collapsed together in summative assessments of well-being. On a chronological timescale, the number of agonistic behaviours, in the form of threat displays, steadily declined until approximately day 100, before reaching a plateau. This implies that a significant fall in agonistic behaviours may be a more reliable indicator of training progress and habituation or reduced frustration (at not getting the rewards on-demand) than cortisol profiles alone.

Animal welfare implications

As has been demonstrated in other species (Moberg 2000), these results serve to emphasise that cortisol responses alone should not be used as an index of a baboon's welfare. The current study is unique in that both physiological and behavioural responses to PRT are described. This approach enabled comprehensive monitoring of animal progress during training and increased our understanding of the impact of PRT on animal welfare.

Acknowledgements

This research was supported by the Zoological Parks Board of NSW, Royal Prince Alfred Hospital and Australian Research Council. The authors are grateful to L Batt for valuable assistance with the study including data management and preliminary analyses, and to M O'Donnell for early statistical advice. A Birrell, A Hennessy, G Evans and WMC Maxwell are thanked for their support of this research. D Lewis, K Heasman, A Peel, J Whitney and V Weir are thanked for their technical assistance.

References

Bell B and Khan P 2001 Training multi-task medical behaviours in the bonobo (*Pan paniscus*) In: *The apes, challenges for the 21st century* pp 128-130. 10-13 May 2000, Brookfield Zoo, Chicago Zoological Society, Illinois, USA

Bloomsmith MA, Laule GE, Alford PL and Thurston RH 1994 Using training to moderate chimpanzee aggression during feeding. *Zoo Biology 13*: 557-566

Bloomsmith MA, Stone AM and Laule GE 1998 Positive reinforcement training to enhance the voluntary movement of group-housed chimpanzees. *Zoo Biology 17*: 333-341

Boyce WT, Champoux M, Suomi SJ and Gunnar MR 1995 Salivary cortisol in nursery-reared rhesus monkeys: reactivity to peer interactions and altered circadian activity. *Developmental Psychology 28*: 257-267

Breland K and Breland M 1961 The misbehaviour of organisms. *American Psychologist 16*: 681-684

Brown CS and Loskutoff NM 1998 A training program for noninvasive semen collection in captive western lowland gorillas (*Gorilla gorilla gorilla*). *Zoo Biology 17*: 143-151

Cooper JJ 1998 Comparative learning theory and its application in the training of horses. *Equine Veterinary Journal 27*: 39-43

Cross N, Pines MK and Rogers LJ 2004 Saliva sampling to assess cortisol levels in unrestrained common marmosets and the effect of behavioral stress. *American Journal of Primatology 62*: 107-114

Desmond T and Laule G 1994 Use of Positive Reinforcement Training in the Management of Species for Reproduction. *Zoo Biology 13*: 471-477

Estes R 1991 *The Behaviour Guide to African Mammals: including Hoofed Mammals, Carnivores and Primates*. University of California Press: Berkeley, USA

Fuchs E, Kirschbaum C, Benisch D and Bieser A 1997 Salivary cortisol: a non-invasive measure of hypothalamo-pituitary-adrenocortical activity in the squirrel monkey, *Saimiri sciureus*. *Laboratory Animal 31*: 306- 311

Hastie T and Tibshirani RJ 1990 *Generalised Additive Models.* Chapman & Hall: London, UK

Hemsworth PH and Barnett JL 2000 Human – Animal Interactions and Animal Stress. In: Moberg GP and Mench JA (eds) *The Biology of Animal Stress – Basic Principles and Implications for Animal Welfare* pp 309-335. CABI Publishing: New York, USA

Insightful Corporation 2001 *S-PLUS 6 for Windows Guide to Statistics, Volume 1*. Insightful Corporation: Seattle, WA, USA

Kirschbaum C and Hellhammer DH 1989 Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology 22*: 150-169

Kobert M 1998 Operant conditioning as an enrichment strategy at the San Diego Zoo. In: *Proceedings of the Third International Conference on Environmental Enrichment* pp 230-236. 12-17 October 1997, Orlando, Florida, USA

Kuhar CW, Bettinger TL and Laudenslager ML 2005 Salivary cortisol and behaviour in an all-male group of western lowland gorillas (*Gorilla g. gorilla*). *Animal Welfare 14*: 187-193

Kummer H 1968 Social organisation of hamadryas baboons, a field study. *Bibliotheca Primatologica 6*: 1-188

Lambeth SP, Hau J, Perlman JE, Martino M and Schapiro SJ 2006 Positive reinforcement training affects hematologic and serum chemistry values in captive chimpanzees (*Pan troglodytes*). *American Journal of Primatology 68*: 245-256

Laule GE, Thurston RH, Alford PL and Bloomsmith MA 1996 Training to reliably obtain blood and urine samples from a young diabetic chimpanzee (*Pan troglodytes*). *Zoo Biology 15*: 587-591

Laule G and Desmond T 1998 Positive reinforcement training as an enrichment strategy. In Shepardsen DJ, Mellen JD and Hutchins M (eds) *Second Nature: Environmental Enrichment for Captive Animals* pp 302-313. Smithsonian Institution Press: Washington, DC, USA

Laule GE, Bloomsmith MA and Schapiro SJ 2003 The use of positive reinforcement training techniques to enhance the care, management and welfare of primates in the laboratory. *Journal of Applied Animal Welfare Science 6*: 163-173

Laule G and Whittaker M 2007 Enhancing nonhuman primate care and welfare through the use of positive reinforcement training**.** *Journal of Applied Animal Welfare Science 10*: 31-38

Lutz CK, Tiefenbacher S, Jorgensen MJ, Meyer JS and Novak MA 2000 Techniques for collecting saliva from awake, unrestrained adult monkeys for cortisol assay. *American Journal of Primatology 52*: 93-99

Mason JW 1968 A review of psychoendocrine research on the pituitary-adrenal cortical system. *Psychosomatic Medicine 30*: 576-607

McEwen BS and Sapolsky RM 1995 Stress and cognitive function. *Current Opinion in Neurobiology 5*: 205–216

McGreevy PD and Boakes RA 2007 *Carrots and Sticks: Principles of Animal Training*. Cambridge University Press: Cambridge, UK

Moberg GP 2000 Biological Response to Stress: Implications for Animal Welfare, In: Moberg GP and Mench JA (eds) *The Biology of Animal Stress – Basic Principles and Implications for Animal Welfare* pp 1-21. CABI Publishing: New York, USA

Morelius E, Nelson N and Theodorsson E 2006 Saliva collection using cotton buds with wooden sticks: a note of caution. *Scandinavian Journal of Clinical and Laboratory Investigation 66*: 15-18

Munck APM, Guyre PM and Holbrook NJ 1984 Physiological functions of glucocorticosteroids in stress and their relation to pharmacological actions. *Endocrine Reviews 5*: 25–44

Pryor K 1975 *Lads before the wind: diary of a dolphin trainer*. Harper & Row: New York, USA

Rubin L, Oppegard C and Hintz HF 1980 The effect of varying the temporal distribution of conditioning trials on equine learning behaviour. *Journal of Animal Science 50*: 1184–1187

Sapolsky RM 1990 Adrenocortical function, social rank and personality among wild baboons. *Biological Psychiatry 28*: 862–878

Savastano G, Hanson A and McCann C 2003 The development of an operant conditioning training program for new world primates at the Bronx Zoo. *Journal of Applied Animal Welfare Science 6*: 247-261

Schapiro SJ, Bloomsmith MA and Laule GE 2003 Positive reinforcement training as a technique to alter nonhuman primate behaviour: quantitative assessments of effectiveness. *Journal of Applied Animal Welfare Science 6*: 175-187

Thorndike EL 1898 *Animal Intelligence: An Experimental Study of the Associative Processes in Animals (Psychological Review, Monograph Supplements, No 8)*. Macmillan Press: New York, USA

Vining RF, McGinley RA, Maksvytis JJ and Ho KY 1983 Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. *Annals Clinical Biochemistry 20*: 329-335

https://doi.org/10.1017/S0962728600027639 Published online by Cambridge University Press