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Stress, the HPA axis, and nonhuman primate well-being: A review

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Abstract

Numerous stressors are routinely encountered by wild-living primates (e.g., food scarcity, predation, aggressive interactions, and parasitism). Although many of these stressors are eliminated in laboratory environments, other stressors may be present in that access to space and social partners is often restricted. Stress affects many physiological systems including the hypothalamic-pituitary-adrenocortical (HPA) axis, which is the focus of this review. The glucocorticoid, cortisol, is the ultimate output of this system in nonhuman primates, and levels of this hormone are used as an index of stress. Researchers can measure cortisol from several sampling matrices that include blood, saliva, urine, faeces, and hair. A comparison of the advantages and disadvantages of each sampling matrix is provided to aid researchers in selecting an optimal strategy for their research. Stress and its relationship to welfare have been examined in nonhuman primates using two complimentary approaches: comparing baseline cortisol levels under different conditions, or determining the reactivity of the system through exposure to a stressor. Much of this work is focused on colony management practices and developmental models of abnormal behaviour. Certain colony practices are known to increase stress at least temporarily. Both blood sampling and relocation are examples of this effect, and efforts have been made to reduce some of the more stressful aspects of these procedures. In contrast, other colony management practices such as social housing and environmental enrichment are hypothesized to reduce stress. Testing this hypothesis by comparing baseline cortisol levels has not proved useful, probably due to “floor” effects; however, social buffering studies have shown the powerful role of social housing in mitigating reactions of nonhuman primates to stressful events. Models of abnormal behaviour come from two sources: experimentally induced alterations in early experience (e.g., nursery rearing), and the spontaneous development of behavioural pathology

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(e.g., self-injurious behaviour). Investigators have often assumed that abnormal behaviour is a marker for stress and thus such monkeys are predicted to have higher cortisol levels than controls. However, an emerging finding is that monkeys with abnormal behaviour are more likely to show a pattern of lowered cortisol concentrations which may reflect either an altered set point or a blunting of the stress response system. These findings parallel human clinical studies demonstrating that neuropsychiatric disorders may be associated with either increased or decreased activity of the HPA system, depending on the aetiology and manifestation of the disorder and their potential influence in provoking allostatic shifts in system functioning.

Keywords

Stress; HPA axis; nursery rearing; social housing; environmental enrichment; cortisol

1. Introduction

It is commonly assumed that laboratory environments are stressful for nonhuman primates, in part because of spatial and social constraints. For example, rhesus monkeys typically live in large socially complex troops with large home ranges whereas in the laboratory, they are often maintained in relatively smaller environments with limited companionship (usually pair housing). However, life in nature is also stressful. Free-ranging rhesus monkeys routinely experience stressful situations that include severe aggression during the breeding season, dominance interactions, disease, parasitism, predation, and food shortages leading to food competition (Aureli et al., 1999; Beehner et al., 2005; Sapolsky, 1987). Thus, stress is a ubiquitous feature of primate life whether in the laboratory or in the wild. The goals of this article are to briefly review the concept of stress, examine and evaluate the various ways to measure stress with a specific focus on the hypothalamic-pituitary-adrenocortical (HPA) axis, and identify possible relationships between stress and well-being in laboratory housed nonhuman primates.

1.1. What is Stress?

Stress can be defined as a perturbation of an organism's physiological and/or behavioural homeostasis as a result of exposure to certain events or situations (termed stressors). Perturbations can occur in response to rewarding events (called eustress); however, researchers have focused much more intensively on perturbations resulting from aversive events (called distress) (Selye, 1975). What constitutes an "aversive" event for an animal is often determined by how we assess the event, since self-report by nonhuman primates is not possible.

In many instances, the perturbation (hereafter referred to as the stress response) is brief and homeostasis is restored. In other instances, the stressor may be chronic and homeostasis is not restored, producing physiological dysregulation (i.e., recurring stress responses). Alternatively, a persistent challenge to homeostasis may, over time, lead to allostasis, which is a readjustment of the physiological system in which the homeostatic baseline has been shifted to account for the changing conditions (e.g., increased basal cortisol levels in the absence of a stressor) (Sterling and Eyer, 1988). But allostasis may inflict its own cost in terms of increased energy demands on the organism. This increased cost or demand (sometimes conceptualized as "wear and tear" on the body) has been termed allostatic load (McEwen, 1998; Stewart, 2006).

1.2. Stress and the HPA axis

The HPA axis is one of many systems that are activated during exposure to stressful events. Because activation results in the production of cortisol, the primary glucocorticoid of human and nonhuman primates alike, the concentration of this hormone is often used as an index of the stress response (O'Connor et al., 2000). Two different approaches can be taken in assessing HPA axis activity. In the first approach, baseline samples of cortisol are collected to answer questions such as what is the relative “stressfulness” of different housing conditions. In this case, researchers measure cortisol levels in animals living under these different conditions without imposing the subjects to any physiological or behavioural challenge. Two key factors determine the validity of the data obtained from such studies. First, the animals must be well adapted to their respective living conditions for the comparison to be valid. Second, collection of the biological samples from which the cortisol will be measured must be minimally disruptive, otherwise the collection procedure itself may produce stress and skew the data (see discussion below on plasma/serum sampling). In the second approach, a stressor is imposed on the subjects to determine the HPA axis response to the challenge. Because nonhuman primate research is frequently limited with respect to availability of animals, stress response studies are often performed using a within-group design in which every subject contributes one or more baseline (i.e., pre-exposure) samples and one or more stress (i.e., during- or post-exposure) samples for cortisol measurement. The stressor may be relatively short, lasting for minutes (e.g., injections), an hour (e.g., exposure to novelty or brief separations from companions), or it may involve longer time periods of months or more in which the effects of exposure to an environmental change are examined (e.g., transfer from social to individual cage housing). Regardless of the time frame, the relevant comparison is how the cortisol levels in individual animals changed in response to the stressor.

One of the traditional ways of measuring the stress response has been to assay cortisol in plasma or serum, with the later advent of salivary measurement. These procedures are particularly useful for measuring the acute (minute by minute) response to a stressor. In recent years, however, researchers have focused on how organisms cope and adapt to long-term stressors. In animals, this effort is concentrated on improving welfare both by increasing the quality of captive environments for laboratory animals and by assessing the impact of ecological factors such as industrial pollutants on free-ranging animals. In humans, there is considerable interest in understanding the role of the HPA axis in the aetiology of neuropsychiatric disorders and in examining the effects of long-term stressors such as unemployment and ecological disasters (e.g., earthquakes and tsunamis) on HPA axis activity. The recently developed technique of measuring cortisol in hair has revolutionized our ability to assess long-term HPA activity and to use this information to evaluate the effects of chronic stress exposure (Davenport et al., 2006).

2. Methodological issues in measuring the HPA stress response

Cortisol can be obtained from many different fluid compartments and body excreta. Today the laboratory scientist and field researcher have several options for measuring stress responses in nonhuman primates that include assaying cortisol in serum/plasma, saliva, urine, faeces, and hair. Each of these sampling matrices has advantages and disadvantages, and these must be considered in determining which sampling matrix is best for answering the research questions at hand, such as evaluating the effects of captive environments, identifying the presence of disease, and/or determining the consequences of an experimental manipulation. In Table 1, we present some of the relevant variables for consideration. The table includes such factors as time frame of measurement, invasiveness of the sampling procedure, relative stability of cortisol in that particular sample matrix (lower values representing less stability), whether the obtained values are influenced by the circadian

rhythm of HPA axis activity, and whether the sample matrix reflects total cortisol content or only the free fraction (i.e., cortisol not bound to plasma proteins).

2.1. Plasma/Serum

The earliest measurements of cortisol in nonhuman primates were obtained using blood samples (Bowman and De Luna, 1968 in rhesus monkeys; Brown et al., 1970 in squirrel monkeys; Hill et al., 1967 in vervet monkeys; and Layne et al., 1964 in chimpanzees). Since that time, plasma/serum cortisol has often been used as an index of the stress response in nonhuman primates and has been shown to vary significantly in animals reared in different social environments (Capitanio et al., 2005; Higley et al., 1992) or maintained under different feeding demands (Champoux et al., 2001). In these instances, the assumption is that chronic stress exposure has resulted in an increased allostatic load which can, depending on the circumstances, be reflected in either an increased or decreased HPA axis set point. More commonly, plasma/serum cortisol has been used to determine acute reactions of animals to short-term stressors using a pre-exposure vs. post-exposure sample comparison (e.g., social separation: Higley et al., 1992; Meyer et al., 1975). In these instances, the assumption is that the stress response is briefly activated and then, when the stressor is removed, homeostasis is restored. Recently, plasma/serum cortisol concentrations have been used to assess long-term stressors (e.g., relocation) where the stressor is not removed but animals may undergo some adaptation (Davenport et al., 2008).

There are many issues in assaying cortisol in blood that can make interpretation of data challenging. First, the procedures for obtaining blood samples usually involve some combination of physical restraint and sedation unless the animal has been fitted with an indwelling venous catheter or has been trained to offer a limb for sample collection (Coleman et al., 2008; Lambeth et al., 2005). Restraint and sedation are generally stressful to the animal, thereby making it difficult to obtain accurate baseline levels of cortisol unless the procedures are conducted extremely rapidly. Second, a single plasma/serum sample only represents the stress response at a particular moment in time. To obtain better estimates and reduce random variation, multiple samples at the same time point across several days might be required. Complicating the picture is the very pronounced circadian rhythm in cortisol secretion with a peak in the early morning shortly after light onset (7:00-8:00 h) and a trough some hours after light offset (between 23:00-4:00 h). Because recent research has shown a flattening of the cortisol rhythm in monkeys exposed to adverse early rearing (Sanchez et al., 2005), it may be important to collect several samples across the day to more fully evaluate HPA axis activity. Finally, assessments of cortisol in plasma measure total levels, which include both the free and the bound portions. Total cortisol is an appropriate measure of entire adrenocortical output, but because both acute and chronic stress can alter circulating concentrations of corticosteroid binding globulin (CBG) (Beishuizen et al., 2001; Davenport et al., 2008) and because only the free fraction is thought to be biologically active (although see Levine et al., 2007), there can be some benefit to choosing a sample matrix that only measures the free fraction of plasma cortisol.

2.2. Saliva

In recent years, saliva sampling has provided an important alternative to blood sampling, particularly in human studies (Hellhammer et al., 2009). This approach was first adapted for use in nonhuman primates by Boyce et al. (1995), who measured salivary cortisol in infant rhesus monkeys housed in a nursery setting. We subsequently developed a procedure for collecting saliva from awake, unrestrained adult male rhesus monkeys (Lutz et al., 2000), that was then adapted for use with squirrel monkeys (Tiefenbacher et al., 2003). Most recently, Higham et al. (2010) reported a method for collecting saliva samples for cortisol measurement from free-ranging rhesus monkey on the island of Cayo Santiago, Puerto Rico

As in blood sampling, cortisol obtained from saliva is a point sample that is also subject to circadian variation. Thus, some of the issues mentioned above with blood sampling also pertain here.

There are advantages to measuring salivary as compared to plasma/serum cortisol. First, salivary cortisol is generally thought to reflect the biologically active free fraction. Second, nonhuman primates can be trained to willingly chew on a dental rope for sample collection which overcomes the problem of using stressful procedures such as sedation and restraint. Nevertheless, some monkeys are difficult to train, requiring many more sessions than other subjects (such variability is also present when training monkeys for non-stressful blood collection; see Coleman et al., 2008 where only six of eight monkeys were trained). Furthermore, even with prior training, the process of “voluntary” sample collection may not be entirely stress-free whether applied to saliva, blood (see above), or urine (see next section).

A major limitation to the use of salivary cortisol with nonhuman primates is that much less information is available about circadian variation and the time course of the salivary cortisol responses to different stressors. In humans, the timing of the peak salivary cortisol response to a mild stressor is similar to that found in plasma cortisol (Kudielka et al.; 2004). At present, no such comparisons exist in the nonhuman primate literature. However, Heintz et al. (2011) recently showed that the salivary cortisol response of captive chimpanzees to the challenge of an intramuscular adrenocorticotrophic hormone (ACTH) injection peaked at approximately 45 min, which is similar to the salivary response in humans. Nevertheless, such time course information on salivary cortisol responses to stress is generally lacking for most species of nonhuman primates. A second disadvantage is the possibility of blood contamination from gum infections which will yield artificially high cortisol concentrations in individual monkeys. A final concern is low saliva production in some animals, a condition that we refer to as “dry mouth.” In one study involving collection of saliva from rhesus monkeys during health exams in which they were anesthetized, about 10% of the monkeys produced insufficient saliva volumes for assay. A comparison of their plasma cortisol values revealed that monkeys with dry mouth had significantly higher levels of plasma cortisol than monkeys that produced enough saliva for assay (Davenport et al., 2003). Salivary cortisol and plasma cortisol levels were highly correlated in the latter group of animals. Thus, it is possible to lose a subset of subjects that differ from the overall population.

2.3. Urine

Measuring cortisol in urine is sometimes used as an alternative to plasma or saliva sampling. With respect to nonhuman primates, cortisol was first measured in the urine of cebus monkeys by Birchall et al. (1966). It is important to note that more than 50% of cortisol excreted in the urine is in a conjugated form, mostly with sulfate and glucuronic acid (Bahr et al., 2000). There are relatively simple enzymatic procedures to remove the conjugated groups, thereby permitting the measurement of total excreted cortisol (free plus conjugated). Alternatively, one may omit the enzymatic step and specifically measure urinary free cortisol. We are not aware of any particular advantage to one approach over the other unless one is concerned with the possibility that one’s experimental manipulation could have influenced the rate of cortisol conjugation prior to entry into the urine. Another issue that must be taken into account is individual differences in daily urine output, which can confound interpretation of the data (e.g., a certain amount of cortisol excretion into a large urine volume will yield a lower cortisol concentration than the same amount excreted into a small urine volume). This potential confound is usually corrected by assessing creatinine levels in each sample (creatinine, which is a product of muscle metabolism, is excreted at a

relatively steady rate independently of cortisol excretion) and then dividing the cortisol value by the corresponding creatinine value.

Several different methodological approaches are used by investigators who wish to assess HPA activity by measuring urinary cortisol. One possibility is to collect 24-h urine samples to provide a complete picture of cortisol output over that time frame. However, in order to accomplish this goal, the animals must be laboratory housed individually either in metabolism cages specialized for collection of excreta (e.g., Setchell et al., 1977) or at least equipped with some kind of appropriate collection pan (e.g., Tiefenbacher et al., 2004). In the latter case, there is a significant risk of contamination with faecal matter unless a fine mesh screen is used to minimize the ability of droppings to pass through into the pan. A much different approach has been developed for use with group-living New World species such as marmosets and tamarins in which individual animals are trained to allow collection of the first morning void by a familiar investigator who enters the group pen immediately at the time of light onset. Although this approach does not provide for a full 24-h collection, it has still proven quite useful for assessing changes in cortisol output under a variety of experimental conditions (e.g., Dettling et al., 1998; 2002; Ziegler et al., 1996). Urinary cortisol has also been measured in great apes, both in captivity and in the wild. For example, chimpanzees living in indoor-outdoor enclosures have been trained using positive reinforcement to urinate into a cup on command for the purpose of cortisol measurement (Anestis, 2005, 2009; Anestis and Bribiescas, 2004). Urine samples (both morning void samples and others obtained opportunistically) have additionally been obtained from wild living chimpanzees and mountain gorillas (Muller and Lipson, 2003; Robbins and Czekala, 1997).

Because urinary cortisol reflects adrenocortical output over a period of hours up to an entire day, it clearly serves a different purpose than the point samples obtained from plasma or saliva. Circadian variation still plays a role in the obtained data when samples are not collected over a 24-h period. However, unlike plasma or saliva, urinary cortisol levels are not subject to the potential stress of sample collection nor are they confounded by random stressful events that may have occurred shortly before the collection time.

2.4. Faeces

The other excretory material from which cortisol can be measured is faeces. Faecal cortisol (and/or metabolite) determinations in free-ranging animals have become an important tool in wildlife conservation (Keay et al., 2006; Millsaugh and Washburn, 2004; Romano et al., 2010). Nonetheless, faecal cortisol can also be measured in captive animals using collection techniques such as those discussed above for urine.

Faeces from nonhuman primates typically contain mixtures of substances including unmetabolised cortisol, corticosterone, and reduced metabolites such as 11-oxo-etiocholanolone and 11 β -hydroxy-etiocholanolone. In a study by Heistermann and co-workers (2006) involving long-tailed and Barbary macaques, marmosets, chimpanzees, and gorillas, stimulation of the HPA axis revealed species differences in which substance(s) exhibited a robust response to the stimulation. Thus, the choice of substance to measure (i.e., cortisol vs. metabolites) may impact the ability to detect stress responses when using faecal assays with nonhuman primates. The time frame reflected by faecal cortisol levels will vary as a function of species and food availability. Even within a particular species, there are likely individual differences in rates of defecation. Other factors to consider that can affect faecal cortisol levels include time of day of collection, sex, age, and season (particularly with respect to reproductive status of the animals) (Touma and Palme, 2005).

Faecal cortisol and/or metabolite levels have been used in several studies to document changes in stress or well-being in captive or free-ranging nonhuman primates. Other studies have demonstrated relationships between faecal cortisol and such factors as food availability and tourist contact for howler monkeys in Belize (Behie et al., 2010), parasite load in male Ugandan chimpanzees (Muehlenbein and Watts, 2010), pregnancy in semi-free-ranging female mandrills in Gabon (Setchell et al., 2008), periods of dominance rank instability among male mandrills from the same colony (Setchell et al., 2010), and changes in male dominance rank in white-faced capuchins in Costa Rica (when faecal cortisol was measured in the females; Carnegie et al., 2011). These examples illustrate the utility of faecal cortisol as an index of HPA activity and the many factors that influence it in wild-living nonhuman primates.

2.5. Hair

The recently developed technique of measuring cortisol from hair provides a powerful new tool with which to assess chronic HPA axis activity. For many years, hair has been used in forensic toxicology to detect the presence of illicit substances such as anabolic steroids and drugs of abuse (Barroso et al., 2011). However, investigators paid relatively little attention to the possibility of measuring cortisol in hair until Raul et al. (2004) reported that significant concentrations of endogenously produced cortisol and cortisone could be measured in the hair of healthy human subjects. In 2006, we developed a procedure to measure hair cortisol in rhesus monkeys (Davenport et al., 2006). Since that time, hair cortisol has been measured in a variety of species including humans, rhesus monkeys, vervet monkeys, cats, dogs, chipmunks, and polar bears, and significant relationships between hair cortisol and either stress or relevant psychological variables such as anxiety or novelty avoidance have been identified (e.g., see Bechshøft et al., 2011; Davenport et al., 2008; Dettmer et al., 2012; Gow et al., 2010). Taken together, the human and animal findings have validated the use of hair cortisol to assess long-term adrenocortical activity and to relate such activity to stressors and to abnormal behaviour. Among the other advantages of this approach are that the results are unaffected either by the time of day of sample collection or by any stress that could be associated with obtaining the hair (e.g., if animals have to be sedated for this purpose).

However, as a sample matrix for measuring cortisol, hair presents a new set of issues that include: 1) what is the time frame represented by the hair sample and 2) can segments of the hair sample be used as a chronology of events? For establishing the time frame, researchers can obtain estimates of rates of hair growth and develop appropriate time scales or they can shave the hair initially and then collect the regrown hair after a predetermined time period. Given that little is known about hair growth rates in nonhuman primates and that hair growth rates may be subject to wide individual and seasonal variation, the latter experimental approach may be preferred in such species.

The question of using hair as a chronology of events has been mainly evaluated in humans. Human scalp hair is known to grow at the rate of approximately 1 cm per month. Assuming that cortisol molecules don't move significantly within the hair shaft once deposited (which seems to be borne out by existing data), then one may use different parts of the hair sample proximal to distal from the scalp to provide a retrospective measure of cortisol deposition over time (i.e., the 1-cm segment closest to the scalp would reflect deposition over the most recent month, the next 1-cm segment would reflect the next previous month, etc.). Indeed, two studies conducted on pregnant women confirm this idea (D'Anna-Hernandez et al., 2011; Kirschbaum et al., 2009). One limitation to this approach is that repeated washing of hair might leach some of the cortisol from within the hair shaft causing cortisol levels to decline significantly as a function of the distance from the scalp. We recently confirmed this hypothesis using monkey hair samples treated with differing numbers of washes with a

standard commercially available shampoo solution (Hamel et al., 2011). Importantly, a control condition in which hair samples were washed with tap water alone showed nearly the same amount of cortisol loss. Thus, any comparison of cortisol concentrations between free-ranging/corral housed and laboratory housed primates might be confounded by exposure to rainfall, especially if such rainfall is frequent (e.g., in tropical climates).

Other issues include potential sources of contamination and whether or not to wash the hair to remove them, how to process the hair prior to cortisol extraction, how to extract the cortisol, and what method to use to measure the extracted hormone. A detailed discussion of these issues can be found in our methods paper (Davenport et al., 2006). An important feature of this assay involves washing the hair to remove any surface cortisol contamination excreted in sweat or sebum with a mild solvent treatment (two, 3-min isopropanol washes). We have also found that grinding the hair into a powder using a ball mill yields significantly more cortisol recovery than mincing the hair with scissors.

One final point concerns the source of the cortisol measured in the hair sample. There is little doubt that cortisol (presumably from the free fraction) enters hair follicles from the bloodstream, and this has been assumed by most investigators to be the source of the hormone extracted from one's samples. However, in 2005, Ito et al. reported the discovery in human hair of an entire system of HPA axis components (culminating in cortisol synthesis) within the hair follicles themselves. Subsequent studies have shown that hair responds rapidly to localized stressors such as immersion in ice-cold water (Sharpley et al., 2009; 2010). While not disputing the above findings, we strongly believe that they do not invalidate the use of hair cortisol as an index of long-term stress, probably reflecting changes in central HPA activity. First, we and most other investigators are careful to shave the hair, not pluck it. Consequently, the cortisol-synthesizing hair follicles remain in the skin and are not included in the sample. Second, thus far there is no evidence that the follicular system responds to psychological stressors such as those shown to elevate hair cortisol in many human and animal studies. Finally, even if the follicular system is eventually shown to respond to non-local (including psychological) stressors, it may prove to be part of a coordinated response with the central HPA axis. Thus, although investigators should be aware of the existence of a cortisol synthesizing system within hair follicles, it should not prevent researchers from continuing to add to the growing literature on hair cortisol and its relationship to stress and psychological well-being.

3. Stress and well-being in nonhuman primates

The stressfulness of the captive environment, as assessed through HPA axis activity, has been examined in many different contexts. However, much of the research has focused on colony management practices, enrichment, social experiences, and on the relationship between abnormal behaviour and HPA axis activity. Each of these areas differs in terms of the proposed hypotheses and predictions. As we will show below, the HPA axis is influenced by many different factors including variation in early experience and genotype. We shall also see that studies of similar general phenomena sometimes yield different outcomes, and the existence of divergent findings may make it difficult to draw conclusions that will facilitate animal welfare directly.

3.1 Colony Management: Stress Activating Procedures

Colony management practices include activities that may be stress provoking for nonhuman primates and would be predicted to increase cortisol production. This discussion will focus on two common procedures, namely relocations and blood sampling. In these situations, activation of the stress response is usually limited to the time period of the stressor, and cortisol levels decline when the animal is returned to its previous environment or when it has

adapted to the new environment. Additionally, some research has been dedicated to refining these colony management practices to reduce stress. This objective can be facilitated by determining which aspects of these practices actually activate the HPA axis.

3.1.1. Relocation—Monkeys may be relocated briefly for husbandry activities or permanently moved to a new room, moved to a new building, or transferred to a different facility requiring air and/or ground transport. Relocation is associated with many changes in the animal's environment (both social and physical) and not surprisingly, moving animals to different environments appears to be strongly associated with activation of the HPA axis. If the move is temporary, cortisol concentrations generally decline to baseline when the animal is returned to its home cage environment. If the move is permanent, cortisol levels usually decline to pre-move levels as a function of adaptation to the new environment, although the time course of such adaptation may vary under different conditions.

Temporary moves of 2 days or less have been studied in many different species. For three macaque species (i.e., rhesus, bonnet, and cynomolgus), placement of individuals in an unfamiliar room for 1 h resulted in increased plasma cortisol concentrations (Clarke et al., 1988). HPA axis activation was evident even when some features of the environment remained constant. For example, male rhesus monkeys moved to identical cages in a different building had significantly higher plasma cortisol levels 1 h post-move than pre-move (Phoenix and Chambers; 1984). However, HPA axis activation has not been observed in all species. Cynomolgus macaques removed from their home cage and moved to an observation room showed elevated urinary cortisol levels during their first day and night in the new environment (Crockett et al., 1993), whereas pigtailed macaques exposed to the same treatment showed no effects (Crockett et al., 2000). Squirrel and titi monkeys moved from their home cage to a very different environment showed divergent reactions with only the squirrel monkeys showing significant increases in cortisol levels (Hennessy et al., 1995). The factors underlying these species differences in responsiveness to relocation have not yet been determined.

Transport to a different building or facility can also be stress-inducing. Rhesus monkeys moved permanently from one building to another, resulting in dramatic changes in cage size and animal densities, showed increased hair cortisol concentrations that had returned to baseline 1 year later even though severely abnormal behaviour and plasma cortisol concentrations remained elevated (Davenport et al., 2008). Chimpanzees moved from one facility to another showed a significant increase in faecal cortisol followed by a gradual reduction to pre-transport levels at the new facility (Reimers et al., 2007). Bushbabies responded to a move from one university to another with significant increases in faecal cortisol levels, which returned to baseline 7 days later (Watson et al., 2005). A recent simulation suggests that adaptation may even occur during transport. Cynomolgus macaques, housed in a transport box for 48 h, displayed a significant increase in urinary cortisol that declined over the 48-h time period to levels similar to baseline (Fernstrom et al., 2008). However, the simulation was limited to confinement and did not include transport.

3.1.2. Plasma sampling/venipuncture—Blood sampling procedures, which generally involve capture, restraint, and sedation, result in activation of the HPA axis. When the blood sampling procedures involve capture and restraint without sedation, the HPA axis response can be prolonged. Ninety minutes after capture and initial sampling, plasma cortisol levels remained elevated for rhesus monkey females (Blank et al., 1983) and 24 h following capture and venipuncture, plasma cortisol levels were still elevated in rhesus monkey males (Rose et al., 1978). Even when the animals were sedated for blood sampling, plasma cortisol levels were elevated later that day and during the following night, only returning to baseline the following day (cynomolgus macaques: Crockett et al., 1993; pigtailed macaques:

Crockett et al., 2000; squirrel monkeys: Gonzales et al., 1982; chimpanzees: Whitten et al., 1998). Despite this initial reaction, rhesus monkeys can show rapid habituation to the procedure, showing no cortisol response on the second or third sampling (Mason et al., 1957). However, repeated sampling does not always yield habituation. Squirrel monkeys subjected to repeated blood sampling did not habituate to the procedure even after 5 weeks (Coe et al., 1978).

Blood sampling is a complex procedure with many different factors. Refinement of this procedure so as to reduce stress requires examining these factors and determining their differential contributions to the stress response. One factor is whether the blood sampling occurs in the colony room in view of other monkeys. Watching other monkeys undergo blood sampling appears to be stressful as noted by findings of a positive relationship between the order in which individuals within a colony room were sampled and their plasma cortisol levels (cynomolgus macaques: Flow and Jacques; 1997; rhesus macaques: Meyer et al., 1975). This effect was not observed in monkeys housed in different rooms where they could not view the sampling of other colony roommates (Flow and Jacques, 1997).

Venipuncture itself may not be the most stressful element of blood sampling; removal from the home cage seems to be a significant factor. Venipuncture resulted in a non-significant increase in plasma cortisol for female rhesus monkeys sampled in their home cage, whereas monkeys sampled in a transfer box had a significant increase in plasma cortisol (Line et al., 1987). Similarly, rhesus monkeys sampled in their home cage had a lower response than when sampled in a restraint device (Reinhardt et al., 1991; but see also Reinhardt et al., 1990 for no differential reaction). Furthermore, human handling alone is stressful; squirrel monkeys caught and held in front of their home cage for 30 s and then released had elevated plasma cortisol levels 30 min later (Hennessy et al., 1982).

Continuous blood sampling requires that animals have indwelling catheters with tethering cables attached to jackets. Research suggests that catheterization is in fact the most stressful part of the procedure. Catheterization surgery resulted in increased cortisol levels in female rhesus monkeys, which returned to baseline levels the following day (Socol et al., 1978). Adult cynomolgus macaques fitted with a jacket and an attached cable showed little reaction, whereas catheterization resulted in significant rises in cortisol concentrations (Crockett et al., 1993). Removal of the catheter and jacket resulted in a rapid decline of cortisol levels.

Fortunately, training subjects for aspects of the blood sampling procedure can result in stress attenuation. Monkeys either habituated to the transport box or trained to enter a transport box showed lower stress responses than monkeys that had not been habituated (Dettmer et al., 1996). Stress responses were also reduced in female rhesus monkeys trained to present a hind leg for sampling as compared to untrained monkeys given anaesthesia (Elvidge et al., 1976). Thus, training can be an important tool in mitigating the stress of blood sampling, particularly when blood sampling is a relatively common event thereby insuring maintenance of the “trained state.” However, some animals may be difficult to train and training can be time consuming (see Coleman et al., 2008, in which four of four chimpanzees were successfully trained to present a limb for blood sampling after approximately 6 months, whereas only six of eight monkeys successfully reached criterion).

3.2. Colony management: stress reducing procedures

Considerable attention has been focused on two factors that may reduce the overall stressfulness of the captive environment. The 1991 revision of the Animal Welfare Act, USA emphasizes both the importance of social housing and the value of environmental enhancement in promoting species-typical behaviour and reducing abnormal behaviour. In

contrast to the effects of relocation and blood sampling which increase stress, both social stimulation and environmental enhancement have been hypothesized to reduce stress. However, positive effects may be difficult to identify in animals housed in stable environments, because over time, monkeys are likely to adapt to their environment, thereby making it difficult to detect a reduction in cortisol beyond basal levels. An alternative approach is to examine reactivity of the HPA axis. It is possible that either or both of these variables may be effective in ameliorating stress under conditions where the animals are exposed to a stressor.

3.2.1. Social versus individual housing in adults: basal levels—An important question in this area of research is whether animals housed in individual cages show a dysregulation of the HPA axis compared to socially housed monkeys. This is an extraordinarily complex issue in which a variety of factors play a role including the size of the social group and the species being studied. The effect of the social environment was best demonstrated in squirrel monkeys where females housed in pairs had significantly lower plasma cortisol levels than squirrel monkeys housed individually. However, pair-housed squirrel monkeys also had significantly lower cortisol levels than those housed in larger social groups (Gonzales et al., 1982). In contrast, no difference in cortisol levels was detected between individually housed and pair housed rhesus monkeys (Schapiro et al., 1993). It seems clear that more studies are needed to determine the influence of social versus individual housing on plasma cortisol in the absence of an imposed stressor.

3.2.2. Social buffering: exposure to a stressor—Can a companion ameliorate another animal's response to a stressful situation? Social buffering exists when individuals either experience less overall stress or recover more rapidly from stress in the presence of conspecifics than without (Kikusui et al., 2006). This phenomenon has been most widely studied in primates using separation and stress paradigms in squirrel monkeys. Infant squirrel monkeys separated from their mothers but remaining in the presence of other colony members exhibited lower plasma cortisol in response to the separation compared to infants housed alone (Coe et al., 1978; Levine et al., 1993; Mendoza et al., 1978). Furthermore, infants reared only with their mother in a single cage showed higher reactions to separation than infants reared in social groups with two other mother-infant dyads (Weiner et al., 1987). The same effect has also been observed in juvenile rhesus monkeys where the presence of a single partner was enough to reduce plasma cortisol responses to a novel environment compared to facing the environment alone (Winslow et al., 2003). Similar results were obtained from examining urinary cortisol excretion in marmosets. There was no change in cortisol after 4-day separations from the social group when the individual was placed in close proximity to a pairmate, but significant increases were observed when individuals were alone (Smith et al., 1998). Social buffering can also yield differential outcomes. In adult male squirrel monkeys, the presence of a partner appeared to ameliorate the behavioural response to novelty or to a snake but did not affect the stress response system (Coe et al., 1982). In general, the data show that companions can play a powerful role in ameliorating short-term responses to stressful situations.

3.2.3. Environmental enrichment (EE)—EE is mandated by U.S.A. federal law as a means to promote species-typical behaviour and reduce abnormal behaviour in captive nonhuman primates. Like social housing, EE has been hypothesized to reduce stress. For the reason described above, this hypothesis is actually quite difficult to test. If only basal levels are examined, monkeys are likely to adapt to their environment, and it may be difficult to detect a reduction in cortisol beyond these basal levels (floor effect) when EE is introduced. Additionally, most forms of EE such as toys or foraging devices produce transient behavioural effects that are unlikely to yield sustained changes in cortisol levels. There are

no studies to our knowledge that have looked at whether EE can ameliorate an animal's response to a stressor.

Not surprisingly, therefore, most studies report little if any effect of EE on HPA axis activity. Cortisol levels were not altered in chimpanzees provided with a box containing food and toys (Anestis, 2009), in an orangutan exposed to a newly restructured environment (Pizzutto et al., 2008) or in rhesus monkeys trained to lever-press (Mason et al., 1957), given access to a music box (Novak and Drewsen, 1989), or provided with physical, feeding, and sensory enrichment (Schapiro et al., 1993). No changes in cortisol levels were detected for rhesus monkey infants whose environment was enriched with spring-suspended surrogates and toys compared to non enriched controls (Clarke et al., 1989).

However, other outcomes have occasionally been observed. The hypothesis of stress reduction was confirmed in one study in which male capuchin monkeys given access to a variety of toys and a foraging box showed reduced cortisol levels (Boinski et al., 1999). On the other hand, even the same enrichment procedure with the same investigators can lead to different outcomes. One group of rhesus monkeys given access to a metal music box that dispensed treats showed a reduction in cortisol levels (Line et al., 1991), whereas a second group of mostly female rhesus monkeys did not (Line et al., 1990). Adverse effects are seldom reported; however, adding woodchip litter and forage materials increased urinary cortisol levels significantly in one of the two treated groups of rhesus monkeys (Byrne and Suomi, 1991).

The above findings indicate that there is currently relatively little support for the hypothesis that EE causes a reduction in HPA axis activity or reduces stress levels in nonhuman primates. However, these findings should not be taken to mean that EE has no positive benefits. EE clearly increases species typical behaviour, and in some cases may result in result in a reduction of abnormal behaviour (Lutz and Novak, 2005).

3.3 Stress and models of behavioural pathology

Several lines of research have examined the relationship between stress and the development of pathological behaviour in nonhuman primates. In some of this research, pathological behaviour is induced by specific kinds of early rearing experiences, and efforts are made to understand the biobehavioural consequences of these experiences. Other research is more clinically oriented in that monkeys are studied only after they have spontaneously acquired a disorder (e.g., self-injurious behaviour), and attempts are made to remediate their condition. For both types of research, the assumption has been that adverse early rearing or the acquisition of severely abnormal behaviour is associated with stress and that monkeys should, therefore, show HPA activation and higher levels of cortisol.

3.3.1. Early Rearing Experiences—In the laboratory, monkeys are typically reared in breeding colonies either with mothers and peers or with their mother only. Both of these conditions yield relatively normal developmental outcomes, allowing them to be combined as mother-reared (MR) for the purpose of further discussion. However, infants can also be reared away from their mother in a nursery either for research purposes or for occasional veterinary/medical reasons (e.g., maternal neglect or failure to lactate, infant prematurity or illness).

Two different nursery rearing paradigms have been examined and compared to the more normative MR condition. In the most common procedure, infants are reared continuously with same aged peers (PR) during the first year of life. PR infants develop all relevant species-typical behaviours. However, in contrast to MR infants, PR infants show developmental delays, are more likely to acquire abnormal behaviours (Winslow et al.,

2003), and show hyperemotional behaviour including high levels of clinging, fear, and social withdrawal, which persist throughout life (Capitanio, 1986; Chamove et al., 1973; Champoux et al., 2002; Harlow, 1963; Ruppenthal et al., 1991). This behavioural profile has made the PR condition an important developmental model of anxiety. In the second nursery paradigm, infants are reared continuously with an inanimate surrogate mother and given short (15 min to 2 h) daily play sessions with same-aged peers (SPR) during the first year of life (Sackett et al., 2002; Shannon et al., 1998). SPR monkeys show all relevant species-typical behaviours but unlike PR infants do not develop hyperemotional behaviours. Thus, SPR monkeys more closely resemble MR than PR monkeys (Champoux et al., 1999; Ruppenthal et al., 1991; Sackett et al., 2002; but see Lutz et al., 2007 for a possible vulnerability in some SPR monkeys to self-injurious behaviour). The development of nearly normal behaviour in the SPR condition is crucially dependent on a short daily play period of 2 h or less. A longer play time (e.g., 8 h) results in the transfer of attachment to the playmates and creates a situation in which monkeys are exposed to repeated daily separations. Not surprisingly, this condition, which has been referred to as “intermittent social rearing” results in highly abnormal behaviour (Rommeck et al., 2009b).

Studies of HPA axis activity focused on basal plasma cortisol concentrations in PR and MR infants point to some dysregulation of the HPA axis occurring as a result of the PR condition. However, the nature of the dysregulation and importantly its relationship to stress remains unclear, particularly given that the data do not conform to the hypothesized effects. Indeed, a common finding is lowered plasma cortisol concentrations in PR infants compared to MR infants (Champoux et al., 1989; Shannon et al., 1998; but see also Winslow et al., 2003 for no differences in PR infants compared to MR infants and Feng et al., 2011 for no differences in PR and MR juveniles).

The reactivity of the HPA axis in differentially reared rhesus monkeys has also been examined, and again a review of the data suggests that PR monkeys may show lower cortisol responses. Barr et al. (2004) reported reduced responsiveness to social separation in PR monkeys as compared to MR controls. Consistent with those findings, PR infants were also less responsive than MR infants to a relocation stressor at 6 months of age (Clarke, 1993). In the most comprehensive study to date, PR monkeys showed lower reactions to social separation, a dexamethasone suppression test, and an ACTH challenge than their MR counterparts, leading the investigators to conclude that the lower reactivity in PR infants may reflect an altered set point of the system (Capitanio et al., 2005). However, in a subsequent study at the same facility, no differential reaction to separation, dexamethasone suppression test, and an ACTH challenge was observed in MR and PR monkeys (Rommeck et al., 2011). In this latter study, an intermittent social rearing condition was included, and monkeys exposed to intermittent social pairing and daily separations had significantly lower cortisol levels than either PR or MR monkeys. Other researchers have also failed to detect differences in cortisol responses between MR and PR infants to a brief separation at 1 month of age (Champoux et al., 1989) or to brief separations at 3, 6, and 9 months of age (Shannon et al., 1998). Additionally, heightened reactions in response to separation have been observed in PR compared to MR monkeys at 6 months of age (Higley et al., 1992) along with a delayed reaction to sedation and capture in PR compared to MR juveniles (Feng et al., 2011).

Only a few studies have examined cortisol concentrations in SPR monkeys, but again results are consistent in suggesting that this rearing condition is associated with reduced cortisol levels (compared to MR animals) under various circumstances. In one study in which MR, PR, and SPR infants were compared, both nursery reared groups (PR and SPR) exhibited lower basal levels of plasma cortisol when compared to MR infants (Shannon et al., 1998). SPR infants also showed a reduced response to the stress of a brief separation compared to

MR infants (Meyer et al., 1975; Shannon et al., 1998). Similarly, juvenile (1-3 years old) SPR infants exhibited lower salivary cortisol levels than MR juveniles after capture and sedation for a health exam (Davenport et al., 2003).

One of the limitations of the above work and a possible contributor to the variability of findings is the reliance on plasma cortisol, a point sample that is highly sensitive to events preceding the sample collection. Measuring cortisol in hair provides an opportunity to examine chronic HPA activity, which should be less subject to wide moment-to-moment variability. To our knowledge, only two studies have examined hair cortisol in differently-reared monkeys. But once again the results remain unclear. In the first study, PR and MR monkeys were maintained in their rearing groups until they were relocated to a mixed rearing environment at 7 months of age. Hair cortisol levels were measured at 1½ and 3 years of age, and PR juveniles had significantly lower concentrations of hair cortisol at both time points than MR juveniles (Feng et al., 2010). In a more comprehensive study, Dettmer et al. (2011) examined hair cortisol levels in MPR, PR, and SPR monkeys starting at 6 months through 2 years of age. From birth through 8 months of age, infants remained in their designated rearing group. At 8 months, all monkeys were relocated to a mixed rearing environment where they remained through 2 years of age. Hair cortisol concentrations gradually declined over the first 2 years of life for all monkeys, but the rearing groups exhibited different trajectories across development. At 6 months of age, which was prior to relocation, PR monkeys had significantly higher levels of hair cortisol than both SPR and MR infants. Following the relocation, both nursery rearing groups (SPR and PR) adapted more slowly to the new environment but did not show lower hair cortisol levels. Furthermore, in contrast to the results of Feng et al. (2011), the three groups were physiologically indistinguishable from one another at 2 years of age. Hair cortisol levels obtained at 6 months of age were significantly correlated with anxious behaviour both in the short and longer term in response to the relocation.

Many of the reported effects of peer-rearing appear to be mediated by gene-environment ($G \times E$) interactions, and it is possible that some of the differential findings are the result of the genetic background of the various populations studied. For example, the behavioural and physiological consequences of allelic variation in the serotonin transporter (5-HTT) gene length polymorphism are far more pronounced for PR monkeys than for their MR counterparts (Champoux et al., 2002). PR monkeys carrying at least one copy of the short allele of the 5-HTT gene had higher levels of aggression (Barr et al., 2003) and both lower basal plasma cortisol concentrations and reduced HPA activation following social separation compared to MR monkeys (Barr et al., 2004). In contrast, MR monkeys showed no significant differences attributable to 5-HTT allelic variation in any of these behavioural and physiological measures. Thus, different outcomes may be obtained depending on the prevalence of the short allele in the various groups of PR monkeys.

These findings point to an emerging hypothesis that the nursery rearing environment may produce a dysregulation of the HPA axis consisting of lower basal levels and reduced reactivity to stressors, particularly when combined with certain genotypes. What this might mean and how we interpret these findings with respect to stress are described in the last section. However, the presence of inconsistent findings underscores the need to develop better models for investigating the complex genetic and environmental factors that contribute to HPA axis functioning in differentially reared monkeys.

3.3.2. Effects of Early Maternal Separation and Maternal Abuse—Other factors that can alter HPA axis function during early development include maternal separation and abuse. Marmosets exposed to brief maternal separations from postnatal day 2-28 showed significantly lowered basal urinary cortisol levels on day 28 compared to non-separated

controls (Dettling et al., 2002). Rhesus monkeys exposed to repeated maternal separations did not differ in their overall cortisol concentrations but showed a flattening of the cortisol circadian rhythm in comparison to non-separated controls (Sanchez et al., 2005). These results once again suggest that altered social experiences result in a reduction in the response of the HPA axis.

Contrasting effects of adverse early experiences have also been reported in monkeys that experience naturally-occurring maternal abuse. Abused infant rhesus monkeys showed an elevated plasma cortisol response to a CRH challenge in comparison to non-abused monkeys (Sanchez et al., 2010). However, in a similar CRH challenge, abused marmosets showed a blunted plasma cortisol response (Johnson et al., 1996).

3.3.3. Stress and Self-Injurious Behaviour—Abnormal behaviour in nonhuman primates can take many different forms that include stereotyped movements (e.g., pacing, somersaulting) and self-directed actions (e.g., digit sucking, eye covering). Stereotypic movements are by far the most prevalent in captivity with fewer monkeys showing self-directed anomalies. However, a small percentage (~5-10%) of captive primates can spontaneously develop severely abnormal behaviour (e.g., self-injurious behaviour; SIB), which is a major concern. Although the presence of stereotypic and severely abnormal behaviour is thought to be a manifestation of the stressfulness of the captive environment, there are relatively few studies that have examined this relationship in normally reared adults. Indeed, the largest body of research indicates that the relationship between HPA axis activity and abnormal behaviour is mediated by early-life experiences (e.g., peer rearing as noted above). In this section, we examine a more general premise that spontaneously occurring abnormal behaviour may be linked to a dysregulation of the HPA axis independent of rearing environment, which may also be explained in part by $G \times E$ interactions. Most of this work comes from our laboratory and focuses on SIB in captive adult male rhesus monkeys.

A small percentage (<10%) of monkeys housed in captivity develop a biting disorder in which they bite their limbs (wrists, ankles, knees, upper arm etc.; see Bellanca and Crockett, 2002; Lutz et al., 2003; Rommeck et al., 2009a). This disorder is not restricted to laboratory environments, having been detected both in enriched zoo settings (Novak et al., 2006) and under free-ranging conditions (Grewal, 1981). In the laboratory, the development of SIB is strongly associated with early placement into individual cage housing such that juveniles are at much higher risk for developing SIB than adolescents or adults (Lutz et al., 2003). Monkeys with SIB also show HPA axis dysregulation. Although basal urinary cortisol levels did not differ in monkeys with SIB compared to controls, monkeys with SIB showed a reduced plasma cortisol response to the stress of ketamine sedation (Tiefenbacher et al., 2000) and a blunted cortisol response to an ACTH challenge (Tiefenbacher et al., 2004). In this latter study, the blunted plasma cortisol response was best explained by wounding recency: the more recent the wounding event in monkeys, the more blunted their response was. Although urinary cortisol in response to a dexamethasone challenge did not differ in SIB and control monkeys, it was significantly lower in high frequency biters as compared to low frequency biters.

Taken together, these findings suggest a dysregulation of HPA axis activity which is once again associated with reduced cortisol expression. These findings have been broadened to include other forms of abnormal behaviour. Behavioural assessments were collected on 194 individually-housed male rhesus monkeys for which hair and salivary cortisol concentrations were also measured. Animals with an abnormal behaviour phenotype (showing at least three stereotypic behaviours in a 5-min assessment and/or self-directed biting) exhibited both lower concentrations of hair and salivary cortisol as compared to

normal controls (Kelly et al., 2009). These data suggest a general relationship between a chronic reduction in HPA axis activity and the prevalence of high levels of abnormal behaviour.

Although a finding of reduced HPA axis activity is consistent across studies in our lab, which focuses on rhesus monkeys, this is not the case for the two studies conducted on other species. No relationship between cortisol and abnormal behaviour was detected in long-tailed and pigtailed macaques, although only a relatively few animals exhibited SIB (Crockett et al., 2007). Conversely, SIB in bushbabies was associated with higher levels of plasma cortisol (Watson et al., 2009). Thus, as noted above with respect to rearing condition effects, the relationship between abnormal behaviour and HPA axis activity seems to vary across species.

As reported for early rearing environments, the relationship between HPA axis activity and abnormal behaviour in normally reared monkeys appears to be mediated by $G \times E$ interactions. Miller et al. (2004) reported an association between a single nucleotide polymorphism (SNP) in the rhesus mu-opioid receptor gene (C77G) and cortisol response to ACTH stimulation in a cohort of male rhesus monkeys. Specifically, monkeys with the derived G allele had a lower cortisol response than monkeys with two copies of the C allele. We subsequently showed that males born and raised at our facility with the derived G allele were more likely to have the abnormal phenotype than males with the typical mu-opioid receptor genotype (Kelly et al., 2009). Similarly, Chen et al. (2010) reported that there were associations between 5'- and 3'-regulatory polymorphisms of the tryptophan hydroxylase gene and HPA axis activity and SIB such that the genotype and allele frequencies of -1325Ins>Del differed significantly between wounders and nonwounding controls.

4. Stress Assessment and Colony Health Management

Given the widespread use of cortisol levels as an index of physiological stress, the question arises as to whether cortisol measurements have a role in routine colony management practices. An important objective of primate colony managers and veterinarians is to ensure that the animals under their care enjoy physical health and psychological well-being. The following criteria have been proposed for the assessment of overall health and well-being: (1) the primates are in good physical health for their age and sex, 2) they show a broad range of the species-typical repertoire, 3) they are not in a state of distress, and 4) they can adapt readily to minor changes in their environment (Novak and Suomi, 1998). One might choose to interpret the third criterion as requiring routine cortisol assays of colony animals. However, behavioural assessments of stress are probably more useful at the present time than routinely screening all captive primates for cortisol levels. The reasons for this are many. First the route of sample collection matters. Blood and saliva are point samples and their relationship to long-term stress levels is questionable, and the procedures required for optimal hair processing for cortisol measurement are currently available only in a few labs. Second, in contrast to the metrics available for standard blood tests of red and white cell counts, blood oxygenation, liver enzyme values, and so forth, there are no established norms for ascertaining "abnormal" cortisol levels that require intervention other than either extremely high or extremely low levels associated with primary hyperadrenocorticism or adrenocortical insufficiency respectively. Third, even if we attempted to establish norms for a particular species, there is significant risk that the normative values that emerged would fail to account for all of the variables that can modulate HPA function. For example, there are well established differences in temperament that yield different but normal patterns of cortisol activation and recovery (Suomi, 1999). Finally, as seen in this review, there are significant issues of interpretation; one cannot simply assume that lower cortisol is always good and higher cortisol is always bad. Nevertheless, we acknowledge that measurement of

cortisol (whether in blood, hair, or other matrices) might be a useful adjunct to other on-going assessments for problematic cases in which changes in HPA activity (as an index of altered physiological stress) could be helpful in determining treatment selection and monitoring treatment efficacy.

5. General Conclusions

The preceding discussion has revealed some of the complexities related to using the HPA axis as an index of stress or, conversely, well-being in nonhuman primates. We have summarized the various sample matrices and collection techniques that can be used to study the HPA system, thereby providing investigators with information that should be helpful in choosing the best methods for answering specific research questions. However, it is imperative that researchers keep in mind that this system is only one of many potential indicators of an animal's state of psychological and/or physical health. For example, the other major arm of the physiological stress system, namely the sympatho-adrenomedullary system, has not been discussed since there are far fewer studies making use of sympathetic nervous system or adrenomedullary (e.g., epinephrine or norepinephrine) indices in relation to colony management practices or early rearing procedures in primates. Neither has the scope of this review permitted a discussion of the immune system, which is not only important in most disease states but also communicates with the nervous system in a variety of ways. Finally, behavioural measures obviously constitute a key component of determining the state of an animal's stress or well-being. Such measures should always include abnormal behaviours such as stereotypies and SIB, but a more complete assessment of the animal's behavioural profile is likely to provide a firmer foundation for determining the influence of any experimental manipulation or change in colony management procedures.

With respect to the HPA system, the literature on EE has shown us that it is very difficult to ascertain reductions in stress when animals have been maintained in a largely stable environment to which they are well adapted. In such cases, relatively minor environmental changes such as the addition of new toys, grooming boards, or the like may not have sufficient impact on the stress response system to be discernable in a typical experimental design, particularly if the animals have rapidly habituated to the enrichment device. On the other hand, if the system is challenged by the imposition of a short-term stressor, then it may be possible to show that one's experimental manipulation has resulted in a reduced peak stress response, faster recovery from that peak, or both. For example, housing condition (e.g., social versus individual housing) itself may not result in stress reduction but the presence of a companion can clearly influence stress responsiveness as illustrated by the social buffering phenomenon.

One of the most intriguing findings from the HPA system literature on nonhuman primates is that certain conditions that intuitively seem stressful actually result in reduced activity of the system. Peer-only rearing in macaques is considered to be adverse to the animal's development as evidenced by the long-lasting manifestations of increased anxiety and other abnormal behaviours. Yet, several studies have shown reduced HPA activity (including lower cortisol levels and adrenocortical responsiveness) in peer-reared animals. Monkeys with SIB (which we believe has developmental origins; see Tiefenbacher et al., 2005) and those with a profile of multiple abnormal behaviours similarly exhibit a reduced stress response or, in the latter case, reduced HPA activity as indicated by lower hair cortisol levels. These findings are challenging the widely accepted but simplistic notion that high HPA activity is "bad", whereas low activity is "good" (also see the literature on altered HPA activity in post-traumatic stress disorder; Yehuda, 2009). It seems that adverse early experiences in nonhuman primates can "reset" the HPA axis in some manner. Such a reset

may involve an entire shift in the set-point of the system in which basal levels are reduced as is stress reactivity (set point hypothesis). Alternatively, basal levels may remain relatively unchanged but stress responses are blunted (blunting hypothesis). Only detailed and careful experimental procedures can differentiate between these hypotheses. Moreover, the potential role of allostasis in such shifts remains to be determined. What is clear is that we can no longer simply assume that the level of cortisol in an animal's blood, saliva, urine, faeces, or hair is a direct index of "stress" in the absence of other key information regarding the animal's physiology, behaviour, and environmental context.

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Table 1

Comparison of sampling matrices for cortisol determination

Matrix	Sample Type	Time Frame	Cortisol Fraction	Invasiveness of collection	Sample Stability *	Circadian variability
Blood	Point	Minutes	Total	Modest	1	Yes
Saliva	Point	Minutes	Free	Minimal	2-3	Yes
Urine	State	Hours/day	Free	None	2	Yes**
Faeces	State	Hours/day	Free	None	2-3	Yes
Hair	State	Months	Free	Minimal	5	No

* Lower numbers represent lower stability

** note that circadian variability is not present if 24 h urine sampling is performed.