Endocrinology and Behaviour: A stress-free approach to improving animal welfare

'Happy' animals make better scientific subjects

Following implementation of the UK Animal Procedures Scientific Act (1986) there has been a plethora of research combining endocrine titres with behavioural measures to address applied questions in the field of animal welfare science. The goal of these studies has been to measure and optimize animal welfare. An eloquent example is the reduced welfare observed in collared peccaries (*Pecari tajacu*) as indicated by high glucocorticoid (GC) levels and negative judgment bias in behavioural tests. The latter is associated with space restriction but alleviated by the provision of enrichment[1].

Good animal welfare is essential not only from an ethical standpoint but also to ensure valid scientific outcomes. Animals with good welfare produce more reliable, biologically valid, robust, repeatable scientific data compared to their counterparts with poorer welfare. 'Happy' animals live longer, can be used repeatedly and need replacing less often. This leads to a 'reduction' of animal use and satisfaction of one of the 3Rs: the guiding principles for the use of animals in research[2].

The 'stress' response

Exposure to a physical or psychological 'stressor' causes a cascade of physiological reactions that enable the animal to cope via a dynamic process of change termed allostasis. (There is no uniformly accepted definition of 'stress', but here I define 'stress' as a 'real or perceived threat to homeostasis' with the caveats of uncontrollability and/or unpredictability[3,4]). A primary physiological response to a 'stressor' is activation of the hypothalamic-pituitary-adrenal (HPA) axis and concomitant release of GCs. In the short term GCs trigger adaptive responses that enable the body to cope with the 'stressor' such as mobilizing energy stores. Chronic stress and GC exposure however is extremely damaging. The latter causes, for example, suppressed reproductive and immune function, diminished cognitive ability, impaired tissue repair and compromised neuronal development in the brain; particularly in the pre-frontal cortex. GCs impact other regulatory systems however in addition to those dealing with 'stress' such as the mesolimbic dopaminergic reward system. Rewarding experiences such as feeding, social defeat and consensual sex also trigger GC release[4]. It is thus essential that if titres of GCs are to be used to evaluate 'stress' and inform welfare decisions in animals then additional measures need to be used to correctly interpret GC levels. One quantitative tool, empirically validated as a reliable measurement of 'stress' is behaviour measurement.

Pairing Endocrinology with Behaviour

At the simple end of the spectrum, high levels of GCs co-existing with certain behavioural traits or frequencies, that deviate from those observed in the wild population, such as stereotypic pacing in polar bears (*Ursus maritimus*) are taken to imply that an animal is 'stressed' and potentially experiencing poor welfare[5] These types of data can inform management decisions. In the latter scenario, environmental correlates of high GC and stereotypic pacing in polar bears, such as exhibit size can be modified to improve welfare. This effective pairing of endocrine and behavioural parameters has led to the development of Behavioural Stress Scores (BSS): defined as a set of behaviours, each with an identified sliding scale assumed to indicate varying degrees of 'stress'. In their best form BSS are underpinned and validated by established physiological indicators of 'stress', such as GC titres. For example our laboratory is currently developing a BSS to permit objective non-invasive assessment of welfare in captive amphibians of which thousands are housed in captivity for research, education, captive-breeding and as pets. However, comprehensive, consensually developed Best Practice guidelines for housing and husbandry that provide optimal welfare, are lacking. We are developing a putative scale of behaviours (such as 'walling': rapid swimming back

and forwards along a tank wall) that we have shown to be associated with varying degrees of corticosterone release (which we quantify from the tank water around the subject). In the domestic horse (*Equus ferus caballus*) we have taken this one step further by identifying behaviours for a BSS such as repetitive crib-biting that are correlated with not one but two established physiological indicators of 'stress' i.e. titres of salivary cortisol and heart rate[6]. In this way the pairing of endocrine with behaviour measures can produce reliable, empirically validated, easy to implement measures of animal welfare.

Endocrinology and behaviour - a circular welfare scenario.

Welfare studies that pair endocrinology with behaviour usually extract GC metabolites from sample media that can be collected non-invasively such as urine, faeces or saliva. The reason is that procedures associated with taking a blood sample such as restraint and venepuncture typically raise HPA function and artificially raise GC levels or mask results to experimental treatments. Behaviour theory is implemented to collect these non-invasive samples. Animals are trained using operant conditioning theory i.e. positive reinforcement techniques (PRT) to 'voluntarily donate' their sample. For example trained animals will urinate into a cup upon presentation of a cue – a behaviour shown to take as little as five, thirty minute sessions to train in the golden lion tamarin (*Leontopithecis rosalia*)[7]. Ironically this use of behaviour as a tool to collect endocrine samples (for welfare studies) affects wellbeing itself (possibly via the perception of increased controllability). For example captive common marmosets (*Callithrix jacchus*) involved in PRT programs were better able to deal with routine husbandry stressors such as weighing than their lab partners not involved in such a program[8]. Similarly wolves (*Canis lupus lupus*) participating in PRT had reduced levels of salivary cortisol post-training compared to pre-training[9]. Thus experimental evidence shows that participation in PRT programs improves the welfare of the subjects – the very construct it set out to test.

References

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