Hormones, Stress and Animal Welfare

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Summary
There are numerous endocrine (hormonal) responses during stress and these are often complex. This complexity makes the study of endocrine stress responses challenging and the challenges are intensified when attempts are made to use measures of hormones to assess the welfare of animals because there are so many endocrine systems activated during stress and because there are countless stimuli that trigger these systems. Most research has concentrated on only a small number of these endocrine systems, particularly the hypothalamo-pituitary adrenal axis and the sympathoadrenal system and there is a need to broaden the scope of endocrine systems that are studied. Furthermore, systematic approaches are required to establish when the actions of hormones associated with stress responses result in physiological and/or behavioural consequences that will have negative or positive effects on the welfare of animals.

Introduction
Understanding the welfare of animals requires a comprehensive appreciation of their physiology and behavior. From a physiological perspective it is common to measure hormones that are released during stress and to try and correlate these with the welfare of an animal. This is a tenuous approach at best, not only because it is difficult to achieve a consensus on the definitions of stress and welfare, but also because stress responses are not necessarily associated with impacts on welfare. Welfare has been defined in a variety of ways, perhaps with the biological functioning and affective (emotional) states frameworks being the most commonly accepted (Hemsworth \textit{et al} 2015). Definitions of stress usually refer to disruptions in homeostasis (Tilbrook and Clarke 2006). While this is reasonable, it is important to acknowledge that stress embodies a vast range of physiological responses, including endocrine (hormonal) responses, which are designed to ensure normal bodily function. Inappropriate endocrine responses during stress may have detrimental impacts on this function which may, in turn, negatively impact welfare. Nevertheless, this is not always the case and the endocrine effects may be neutral or even positive.

Currently, there is a poor understanding of the roles of hormones during stress in influencing the welfare of animals. This limits the usefulness of endocrine measures in the assessment of animal welfare. Here, we discuss the reasons for this and suggest an approach to fill this gap in knowledge.

Endocrine responses to stress: complex and varied
Hormonal responses to stress are numerous and complex. A corollary of this is that the discipline is challenging to understand. The challenge is magnified when trying to translate the science to the assessment of animal welfare.

The collective seminal work of Bernard, Cannon and Selye set the platform for our understanding of endocrine responses to stress (Ralph \textit{et al} 2016a). The combined efforts of Bernard and Cannon clearly established that disrupting homeostasis induced a myriad of responses in the body to re-establish the balance. The work of Cannon and Selye identified the sympathoadrenal system and the hypothalamic-pituitary adrenal (HPA) axis, respectively, as frontline physiological systems activated during stress to confront challenges. The catecholamines of the sympathoadrenal system act as neurotransmitters, innervating target tissues, and as hormones, acting throughout the body. The glucocorticoids of the HPA axis are steroid hormones with multiple targets throughout the body. Catecholamines and glucocorticoids have numerous actions, many that stretch way beyond what might be considered the domain of stress hormones. Despite this, these are the most commonly studied hormones in the context of stress responses and, indeed, in the assessment of the welfare of animals.

In addition to the so-called classical stress systems mentioned above, there are numerous peptides that are involved in stress responses, sometimes as regulatory factors, sometimes with direct actions on target tissues. These include corticotrophic releasing hormone, vasopressin, adrenocorticotrophic hormone, the opioid peptides, oxytocin and a number of appetite regulating hormones such as orexin, neuropeptide Y, agouti-related peptide, cocaine and amphetamine regulated transcript, leptin and ghrelin, amongst others (Tilbrook 2007). Another family of peptides, the RF-amides have also been the subject of investigation during stress (Papagiris \textit{et al} 2011) and, recently, we have added to the list by including the actions of the neuropeptides kisspeptin, dynorphin and neurokinin B (Ralph \textit{et al} 2016b).

Coming to terms with such vast endocrine responses is in itself a challenge but the complexity is magnified because there are so many different stimuli that induce stress responses. These stimuli are called stressors and their initiation of the range of stress responses is influenced by the physiological and behavioural state of the animal, sex, experience and genetic and environmental factors (Tilbrook and Clarke 2006).

Acknowledging that endocrine responses are many and varied is important when trying to assess how these responses may impact normal bodily functioning and, further, how this may affect the welfare of an animal. The latter is key to this debate and to understand this we need to know the consequences of the actions of the hormones. We need to appreciate when these actions result in consequences that ensure normal and acceptable bodily function and, in contrast, when the outcomes are physiological and
behavioural effects that are considered to influence the welfare of an animal. This can only be established by systematic research to determine the actions and consequences of the actions of hormones to particular stressors in particular conditions. Such research is lacking in animal welfare science but an example of this systematic approach exists in our research to determine the impact of the glucocorticoid cortisol in causing stress-induced suppression of reproduction in female sheep.

**Systematic determination of the impact of endocrine responses on physiology and behaviour: reproduction case study**

We undertook a series of systematic studies in ewes to establish the importance of cortisol in mediating the inhibitory effects of psychosocial stress on reproduction (Ralph et al 2016b). These studies determined the conditions under which cortisol has an impact, and the consequence of that impact, on each of the (i) tonic secretion of gonadotrophin releasing hormone (GnRH), the regulator of the synthesis and secretion of luteinising hormone (LH), which is necessary for follicular development, (ii) the surge secretion of GnRH and LH that are necessary for ovulation and (iii) sexual behaviour. Thus, we methodically partitioned the reproductive axis. Our strategy was to systematically establish whether cortisol is both sufficient and necessary to suppress reproductive hormone secretion and inhibit sexual behavior. A key innovation in the approach was establishing whether cortisol was necessary for stress-induced inhibition. In other words, were the effects of stress due to cortisol beyond doubt?

An essential element of this strategy was the use of an *in vivo* neuroendocrine model in the female sheep that allowed full quantification of reproductive hormone secretion from the brain to the gonad, the ability to map the specific neuronal populations and pathways that control neuroendocrine function, and to quantify sexual behavior.

This research showed that cortisol is necessary to inhibit some, but not all, aspects of reproduction in female sheep. For example, psychosocial stress inhibits sexual motivation and sexual receptiveness in ewes but cortisol is only responsible for the effect on receptiveness and not motivation. The actions of cortisol during stress vary with reproductive state and there are important interactions with gonadal steroids.

Our approach determined the critical concentrations of circulating cortisol needed to cause particular inhibitory effects, and the sites and mechanisms of action of cortisol to inhibit reproductive events. Thus, it is now possible to predict the conditions under which cortisol synthesis during stress will have an inhibitory impact on reproduction in ewes, what the inhibitory impact will be and, in contrast, when cortisol will not have an inhibitory effect.

We propose that a similar systematic approach to that used to establish the role of cortisol in stress-induced inhibition of reproduction in female sheep would be beneficial in animal welfare science. There needs to be development of models to assess when the actions of particular hormones that are associated with stress responses have consequences that impact the welfare of animals (Figure 1). The objective should be to take this research to a level where reasonable extrapolations can be made about how the measurement of hormones in certain conditions in response to particular challenges will affect the welfare of animals. The approach must be broad and must extend well beyond the classic stress hormones: the glucocorticoids and catecholamines.

**Conclusion**

It is common to measure hormones in studies of stress and welfare in animals, particularly the glucocorticoids and catecholamines. Much of this research has been inconclusive and this is largely due to the challenges associated with understanding the complexity of endocrine systems, the complexity of stress and the link, or otherwise, between endocrine stress responses and the welfare of animals. Furthermore, much research has been narrowly focused on the sympathoadrenal system and HPA axis with insufficient consideration of the other numerous endocrine and neuroendocrine systems that are involved in stress responses and the welfare of animals. Animal welfare science requires systematic research to establish the conditions under which stress results in endocrine actions that impact the welfare of animals. This promises to provide one of the most substantial advances in our understanding of stress and animal welfare.

**References**


Ralph CR and Tilbrook AJ (2016a) *Journal of Animal Science* 94 1-14

