INVITED REVIEW:
The usefulness of measuring glucocorticoids for assessing animal welfare

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ABSTRACT: Glucocorticoids (corticosterone in birds and rodents and cortisol in all other mammals) are glucoregulatory hormones that are synthesized in response to a range of stimuli including stress and are regularly measured in the assessment of animal welfare. Glucocorticoids have many normal or non-stress-related functions, and glucocorticoid synthesis can increase in response to pleasure, excitement, and arousal as well as fear, anxiety, and pain. Often, when assessing animal welfare, little consideration is given to normal non-stress-related glucocorticoid functions or the complex mechanisms that regulate the effects of glucocorticoids on physiology. In addition, it is rarely acknowledged that increased glucocorticoid synthesis can indicate positive welfare states or that a stress response can increase fitness and improve the welfare of an animal. In this paper, we review how and when glucocorticoid synthesis increases, the actions mediated through type I and type II glucocorticoid receptors, the importance of corticosteroid-binding globulin, the role of 11 β-hydroxysteroid dehydrogenase, and the key aspects of neurophysiology relevant to activating the hypothalamo–pituitary–adrenal axis. This is discussed in the context of animal welfare assessment, particularly under the biological functioning and affective states frameworks. We contend that extending the assessment of animal welfare to key brain regions afferent to the hypothalamus and incorporating the aspects of glucocorticoid physiology that affect change in target tissue will advance animal welfare science and inspire more comprehensive assessment of the welfare of animals.

Key words: corticosterone, cortisol, stress responses, welfare assessment

 J. Anim. Sci. 2016.94:457–470
doi:10.2527/jas2015-9645

INTRODUCTION

Glucocorticoids are steroids produced largely by the adrenal glands and are considered to be stress hormones. Stress responses usually involve an increase in glucocorticoid synthesis, so it is common to measure blood concentrations of glucocorticoids when studying stress. Stress has been extensively examined, using a range of strategies and from a diversity of perspectives including the biomedical and animal sciences. The latter embraces measures of glucocorticoids to assist in the assessment of the welfare of animals. The reality is that it is difficult to use measures of circulating glucocorticoids, or any stress hormones, to assess the welfare or health status of an animal, even when these measures are in conjunction with behavioral or other physiological measures. There are many reasons for this, not least that the physiological and behavioral state of an animal, and thereby its welfare, will be impacted by the actions of the stress hormones and that these actions can rarely be evaluated from blood concentrations alone. More information is needed, such as the concentrations, metabolic fate, and actions of hormones within target tissues. The complexity is exacerbated by the vast array of stimuli that induce stress responses. Different categories of stimuli can result in seemingly similar stress responses yet seldom can these be deciphered from peripheral measures. Furthermore, stress hormones rarely act only as stress hormones. A hallmark of the endocrine system is that hormones usually have more than one function (Cawadias, 1940). For example, glucocorticoids influence glucose metabolism through counterregulatory actions (Feldman et al., 1975) that are not necessarily

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Received August 6, 2015.
Accepted November 30, 2015.

categorized as actions of a stress hormone. This area of study is further complicated by the difficulties in establishing accepted definitions of stress and welfare. Here, we review the usefulness of measures of glucocorticoids in the assessment of animal welfare.

Defining Stress

Despite the frequency with which stress is used in everyday language, it is surprisingly difficult to define. It is a term that lends itself to subjectivity and emotiveness and can appear diffuse and lacking in certainty of meaning. We developed a working definition of stress that is "a complex physiological state that embodies a range of integrative and behavioral processes when there is a real or perceived threat to homeostasis" (Tilbrook and Clarke, 2006, p. 285; Tilbrook, 2007).

We acknowledge that this definition is somewhat limiting because stress can embody numerous constructs. Nevertheless, our definition focuses on bodily functions that are adaptive to challenges and allow coping, and this is particularly useful when placing stress responses in the context of the everyday life of an animal. A brief consideration of key aspects of the historic development of the stress concept helps us appreciate that we are dealing with complex systems designed to maintain normal physiological function in the face of challenges called stressors.

The importance of a balanced internal state has long been recognized. As early as 500 to 430 BCE, Greek philosopher Empedocles acknowledged the concept of a steady or harmonious state (Johnson et al., 1992). French physiologist Claude Bernard developed the concept that organisms maintain a stable internal environment, *the milieu interior*, and set the foundation for appreciating the importance of adaptive internal mechanisms that allow organisms to cope with challenges (Johnson et al., 1992). Subsequently, Walter Cannon expanded this theme and coined the term "homeostasis" and described how animals in threatening situations show adaptive responses in which they may fight or retreat. Cannon termed this the "fight or flight" syndrome (Cannon, 1932). It was Cannon's work that established the importance of the sympathoadrenal system in reinstating homeostasis during brief threatening encounters (Cannon, 1932). In the 1930s, Hans Selye developed the general adaptation syndrome, which he defined as "the sum of all nonspecific systemic reactions of the body which ensue with long continued exposure to stress" (Cannon, 1929; Selye, 1946 p. 117, 1955, 1956). Importantly, Selye demonstrated the vital role of the hypothalamo–pituitary–adrenal (**HPA**) axis in adaptation to stressful situations (Selye, 1946).

Irrespective of the definition of stress that one adopts, there is a basic theme that when stressors act on the body there will be a myriad of physiological and behavioral responses that attempt to re-establish homeostasis. This is normal. In addition to re-establishing homeostasis, these physiological and behavioral responses often change physiological set points or enable stability through change. This concept of achieving stability through change is termed allostasis; it allows adaption to a stressor and is adequately reviewed by McEwen and Wingfield (2003). These responses include the activation of various neuroendocrine systems including the sympathoadrenal system and the HPA axis, which produce catecholamines and glucocorticoids, respectively. Both families of hormones are measured in the study of animal welfare but it is the glucocorticoids that have received the most attention.

It has become increasingly common to assess circulating glucocorticoids from measures in media other than blood, such as saliva. Given that, by definition, hormones are transported in blood from their sites of synthesis to their sites of action, we have restricted this discussion to measures in blood (serum or plasma) when assessing peripheral glucocorticoids. We consider that the arguments we pose for blood measures largely apply to media such as saliva.

Defining Animal Welfare

To debate the usefulness of measuring glucocorticoids in assessing animal welfare, it is imperative to establish an acceptable definition of animal welfare. Like the term stress, the term welfare can have many connotations, and therefore, decisions on the acceptable use of animals can involve difficult and complex choices. Consequently, these decisions can be controversial. Simplistically, animal welfare could be considered as how an animal feels right now, but quantification of this is difficult and complex. A definition of animal welfare that has stood the test of time and has provided an agenda for animal welfare science is that of the Brambell Committee that was established by the British Government in 1965 (Brambell et al., 1965). The Brambell Committee introduced the concept that animals should have the freedom to stand up, lie down, turn around, groom themselves, and stretch their limbs. This concept was developed by the Farm Animal Welfare Advisory Committee and became the "five freedoms" approach to animal welfare (Hemsworth et al., 2015). The Brambell Committee defined animal welfare as a wide term that embraces both the physical and mental well-being of the animal while emphasizing the importance of scientific evidence in the evaluation of animal welfare (Brambell et al., 1965). In accordance with this, animal welfare science has developed a multidisciplinary approach to establish methodologies

for evidence-based assessment of the welfare of an animal. As indicated above, these approaches frequently involve an evaluation of stress responses, often with measures of glucocorticoids.

There are several science-based theoretical frameworks for understanding animal welfare including the "biological functioning," "affective states," and "natural living" frameworks. We recently scrutinized these frameworks from the perspective of understanding animal welfare (see Hemsworth et al. [2015] for a review). The biological functioning framework refers to biological activity induced to allow an animal to cope with a challenge and includes physiological responses to stress, involvement of the body repair systems, and immunological defenses as well as a variety of behavioral responses. The affective states framework emphasizes how the capacity of an animal to have emotional experiences influences its welfare. The natural living framework is predicated on the view that the welfare of an animal is improved if it can express normal behavior. Of the 3 frameworks, this is the most poorly defined, with the usefulness of this framework in terms of understanding animal welfare being severely hampered by a lack of definition of, and rationale for, natural behaviors that are desirable or undesirable in terms of animal welfare (Hemsworth et al., 2015). Although the biological functioning and affective states frameworks appear to be the most useful scientific concepts in terms of assessing animal welfare, both have been criticized for limitations: the former, for example, for not adequately including emotions and the latter for the inability to directly measure affective states and to relate these to physiological measures (Hemsworth et al., 2015). Indeed, the biological functioning and affective states frameworks were initially seen as competing, but we have recently contended that knowledge of the dynamic interactions between these frameworks is fundamental to managing and improving animal welfare. For instance, the biological functioning framework includes that affective experiences and affective states are products of biological functioning (Hemsworth et al., 2015).

Not surprisingly, a range of physiological measures are commonly collected when assessing biological functioning, including hormones associated with stress responses, such as the glucocorticoids. If one considers the importance of stress responses in healthy animals, then this makes perfect sense, although interpretation of measures of stress hormones in terms of animal welfare is challenging, even when considering normal biological functioning. The challenge is apparently even greater if measures of stress hormones are to be used to assess affective states. This is partly because both negative and positive emotional states can stimulate stress responses and interpretation from simply measuring the end-point hormones is virtually impossible. Ostensibly, we need to know more about the inputs that caused the increase in circulating concentrations of stress hormones before we can assess the welfare of an animal from these measures.

PHYSIOLOGICAL RESPONSES TO STRESS: THE INELUCTABLE INVOLVEMENT OF CATECHOLAMINES AND GLUCOCORTICOIDS

Sympathoadrenal System (Catecholamines)

The sympathoadrenal system consists of the sympathetic nervous system and the adrenal glands. The catecholamines, which include dopamine, epinephrine, and norepinephrine, initiate the actions of the sympathoadrenal system, which evoke rapid neural, endocrine, behavioral, and muscular activity throughout the body (Sawchenko et al., 1999). For example, within the cardiopulmonary system, catecholamines increase cardiac output, increase respiration rate, and redistribute blood flow to the pulmonary organs and those organs necessary for mounting a response to the stressor (Sawchenko et al., 1999). The sympathetic component of the sympathoadrenal system comprises preganglionic neurons that project from the spinal cord to ganglia, where they synapse with postganglionic neurons that project to, and innervate, target tissues (Turner et al., 2012). Preganglionic neurons release the neurotransmitter acetylcholine that stimulates the postganglionic neurons to release norepinephrine directly into the target tissue. In the adrenal arm of the sympathoadrenal system, preganglionic neurons extend from the spinal cord to ganglia in the adrenal medulla, where the terminals appose endocrine cells are called chromaffin cells. Acetylcholine stimulates synthesis of catecholamines in chromaffin cells in the adrenal medulla and the secretion of epinephrine and norepinephrine into the peripheral blood stream. There are species differences in the amount of norepinephrine and epinephrine released into the peripheral circulation from the adrenal medulla (Tilbrook et al., 2008), but both catecholamines have far-reaching endocrine actions in the body.

Hypothalamo–Pituitary–Adrenal Axis (Glucocorticoids)

The HPA axis is one of the physiological systems almost always activated by stress. It consists of the hypothalamus of the brain, the anterior pituitary gland, and the cortex of the adrenal glands (Tilbrook, 2007). Specialized neurons within the paraventricular nuclei of the hypothalamus synthesize hypophysiotropic neuro-

peptides that regulate the HPA axis. These neurons project to the median eminence and release the neuropeptides into the hypophyseal portal blood system (Tilbrook, 2007). In all mammalian species studied, these neurons secrete corticotrophin-releasing hormone (Tilbrook, 2007). In most mammals studied except for the pig, these neurons also secrete arginine vasopressin, whereas in the pig, they secrete lysine vasopressin, and in all non-mammalian vertebrates, they secrete arginine vasotocin (Tilbrook, 2007). Corticotropin-releasing hormone and arginine vasopressin act on corticotrope cells in the anterior pituitary to stimulate the synthesis of peptides that are derived from the pro-peptide proopiomelanocortin (Delitala et al., 1994). These include adrenocorticotropic hormone, β-endorphin, α-melanocyte stimulating hormone, β-melanocyte stimulating hormone, corticotropin-like intermediate peptide, γ-lipotropic hormone, and met-enkephalin (Delitala et al., 1994). Once secreted from the anterior pituitary into blood, adrenocorticotropic hormone acts on the adrenal cortex to stimulate synthesis of glucocorticoids. In birds and rodents, the principle glucocorticoid is corticosterone, and in all mammals other than rodents, it is cortisol.

Glucocorticoids

Glucocorticoids have glucoregulatory actions and widespread effects to mobilize energy stores throughout the body with the objective to re-establish homeostasis (Turner et al., 2012). Glucocorticoids influence the expression of approximately 10% of the genome and targets include genes controlling metabolism, growth, repair, reproduction, and the management of resource allocation (Maciel et al., 2001; Le et al., 2005). With respect to metabolic actions, glucocorticoids can affect the concentration of nonesterified fatty acids, lactate, and glucose and can influence the concentration of plasma insulin while having a variable effect on glucose metabolism (Devenport et al., 1989; Dallman et al., 1993; Fowden et al., 1993; Andrews and Walker, 1999; Remage-Healey and Romero, 2001; Goldstein et al., 2002; Chaves, 2006; Kyrou and Tsigos, 2009; Franko et al., 2010; Chacko et al., 2011; Restitutti et al., 2012). The synthesis of glucocorticoids increases after eating, and they can increase food intake and, in combination with insulin, can increase fat storage (Devenport et al., 1989; Tempel and Leibowitz, 1994). Glucocorticoid secretion is highly variable and is pulsatile with a periodicity of about 90 min in many species including humans (Follenius et al., 1987), cattle (Thun et al., 1981; Echternkamp, 1984), and sheep (Fulkerson and Tang, 1979). Nonetheless, this pulsatility is not clearly evident in other species including pigs (Mormède et al., 2007). Glucocorticoids are synthesized in a diurnal pattern that is governed by zeitgebers including light, among other factors including genetics (Turner et al., 2012). In matutinal species, there is a peak in the morning and a trough during the evening and night, whereas in nocturnal species, there is a peak in the evening and a trough in the morning (Mormède et al., 2007). Many normal functions induce synthesis of glucocorticoids and these functions are necessary for normal body maintenance, growth, and repair (Olsson and Sapolsky, 2006).

Glucocorticoid Receptors: How Glucocorticoids Act

Glucocorticoids classically exert their actions by entering target cells and binding to mineralocorticoid or type I glucocorticoid receptors and glucocorticoid or type II glucocorticoid receptors (Newton, 2000; Wang, 2005; Sorrells and Sapolsky, 2007). The receptors are generally located in the cytoplasm and the glucocorticoid-receptor complex and then translocate to the nucleus to regulate the transcription of target genes (Sapolsky et al., 2000). Type I glucocorticoid receptors generally regulate the basal activity of the HPA axis whereas type II glucocorticoid receptors are occupied when the concentrations of glucocorticoids are much higher, such as during a stress response (Lim-Tio et al., 1997; Lim-Tio and Fuller, 1998). Type I glucocorticoid receptors typically localize to the hippocampus and other brain regions of the limbic brain such as the lateral septum and amygdala as well as hypothalamic sites; type II glucocorticoid receptors have a more extensive distribution, being most abundant in the hypothalamus centrally and in pituitary corticotropes (De Kloet, 2004). Type I glucocorticoid receptors bind glucocorticoids with high affinity whereas type II glucocorticoid receptors bind glucocorticoids with low affinity (Chapman et al., 2013). Type I glucocorticoid receptors are involved in negative feedback during basal activity and type II glucocorticoid receptors are involved in feedback actions during both basal and stress-induced levels of glucocorticoids (Tilbrook and Clarke, 2006).

The actions of glucocorticoids within a cell will vary according to how much hormonal activity is transmitted via type I or type II glucocorticoid receptors, and this will depend on the amount of intracellular free glucocorticoid and the concentration and sensitivity of type I and type II glucocorticoid receptors in the cell (Bamberger et al., 1996; Chrousos and Kino, 2005; Kino, 2007). Because type I and type II glucocorticoid receptor sensitivity may be influenced by a range of physiological conditions including nutritional state, immune status, reproductive status, and seasonal or circadian rhythms, the glucocorticoid signaling system is highly stochastic (Bamberger et al., 1996; Lim-Tio et al., 1997; Lim-Tio and Fuller, 1998; Chrousos and Kino, 2005; Wang, 2005; Kino, 2007). Therefore, the effects of glucocorticoids on the physiol-

ogy of the animal will be influenced by the abundance of type I and type II glucocorticoid receptors and the amount of free glucocorticoid that is within the tissue and able to bind to these receptors. Measurements of the concentrations of glucocorticoids in the blood will not, on their own, provide sufficient information to understand the tissue concentrations of free glucocorticoids and each type of glucocorticoid receptor and, therefore, the actions of glucocorticoids in target tissues. It is these actions that impact physiological and behavioral functions and, in turn, the welfare of animals (Fig. 1).

Corticosteroid-Binding Globulin: Transport and Protection of Glucocorticoids

Under basal conditions, 80 to 90% of glucocorticoids circulate in the blood bound to proteins, predominantly corticosteroid-binding globulin and, to a lesser extent, albumin (Breuner et al., 2003). Corticosteroid-binding globulin binds glucocorticoid with high affinity and low capacity whereas albumin binds with low affinity and high capacity (Breuner and Orchinik, 2002). Glucocorticoids are considered biologically inactive when bound to proteins as it is only when unbound, or free, that the glucocorticoid is able to migrate from blood to the intracellular environment (Bright, 1995). Therefore, it has been suggested that to study the biological action of glucocorticoids, it is important to delineate between the free and bound fractions (Perogamvros et al., 2012). Nonetheless, it needs to be recognized that the binding of glucocorticoids to proteins in the blood is a mechanism to ensure the transport and protection of the steroids (Sapolsky et al., 2000). Bound steroids will not necessarily be unavailable for physiological actions as they can become free at the site of the target cell.

Only free glucocorticoids are able to enter cells and bind to receptors but glucocorticoids bound to corticosteroid-binding globulin can be liberated from corticosteroid-binding globulin and made available. Corticosteroid-binding globulin is synthesized in the liver and this synthesis can be increased in response to glucocorticoid synthesis. For example, in wild sparrows, there is a seasonal change in glucocorticoid concentrations and a seasonal change in corticosteroid-binding globulin such that free glucocorticoid concentrations remain within narrow limits all year (Romero and Remage-Healey, 2000). Likewise, in many species, there is a circadian rhythm in corticosteroid-binding globulin production that regulates free glucocorticoid concentrations throughout the day. Furthermore, corticosteroid-binding globulin synthesis can increase in response to stressors. For example, a rapid release of corticosteroid-binding globulin from the liver of rats has been shown to restrain the effects of acute glucocorticoid synthesis, demonstrating that the liver can synthesize corticosteroid-binding globulin in response to short-term stressors (Qian et al., 2011).

Free glucocorticoid concentrations can vary independently of total glucocorticoid concentrations, with changes in the binding affinity of corticosteroid-binding globulin influencing the amount of free glucocorticoid in plasma (Barnett et al., 1981, 1984, 1985; Bright, 1995; Alexander and Irvine, 1998; Picard-Hagan et al., 2000; Breuner and Orchinik, 2002). Temperature and pH can influence the binding affinity of corticosteroid-binding globulin whereas neutrophil elastase can cleave the corticosteroid-binding globulin/glucocorticoid compound, resulting in a localized increase in free glucocorticoid (Breuner and Orchinik, 2002; Cohen and Venkatesh, 2009; Perogamvros et al., 2012). There are instances where decreased binding affinity of corticosteroid-binding globulin can increase the amount of free glucocorticoid available. For example, in scrapie-affected ewes, there was no difference in total cortisol concentrations yet a decrease in corticosteroid-binding globulin binding affinity resulted in an increase in free cortisol that influenced the physiology of the ewes (Picard-Hagan et al., 2000). This example highlights that consideration of corticosteroid-binding globulin can be informative because in this instance, there was no change in total cortisol but an increase in free cortisol affected the physiology, and perhaps the welfare, of the ewes. Clearly, the extent to which measures of circulating glucocorticoids will be useful in the assessment of the welfare and health status of an animal will be influenced by the amount of glucocorticoids that are safely transported to the sites of action and then liberated from carrier proteins, such as corticosteroid-binding globulin, to affect biological change.

11 β*-Hydroxysteroid Dehydrogenase: Metabolism of Glucocorticoids*

The 11 β-hydroxysteroid dehydrogenase (**11 β-HSD**) enzyme system can interconvert cortisol and cortisone (Tomlinson et al., 2004; Chapman et al., 2013). The 11 β-HSD enzyme has 2 isomers, 11 β-HSD 1 and 11 β-HSD 2, with 11 β-HSD 1 functioning primarily as a reductase, converting inactive cortisone to active cortisol and 11 β-HSD 2 functioning only in the dehydrogenase mode converting cortisol to inactive cortisone (Cohen and Venkatesh, 2009). Distribution of each isomer is tissue specific with key metabolic tissues such as liver, adipose tissue, and skeletal muscle expressing 11 β-HSD 1 whereas 11 β-HSD 2 is expressed in the kidney, colon, and salivary glands (Morgan et al., 2014). The 11 β-HSD system is regarded as a key element of glucocorticoid metabolism (Tomlinson et al., 2004; Chapman et al., 2013). Within tissue, 11 β-HSD 1 can convert inactive cortisone to active cortisol when additional cortisol is re-

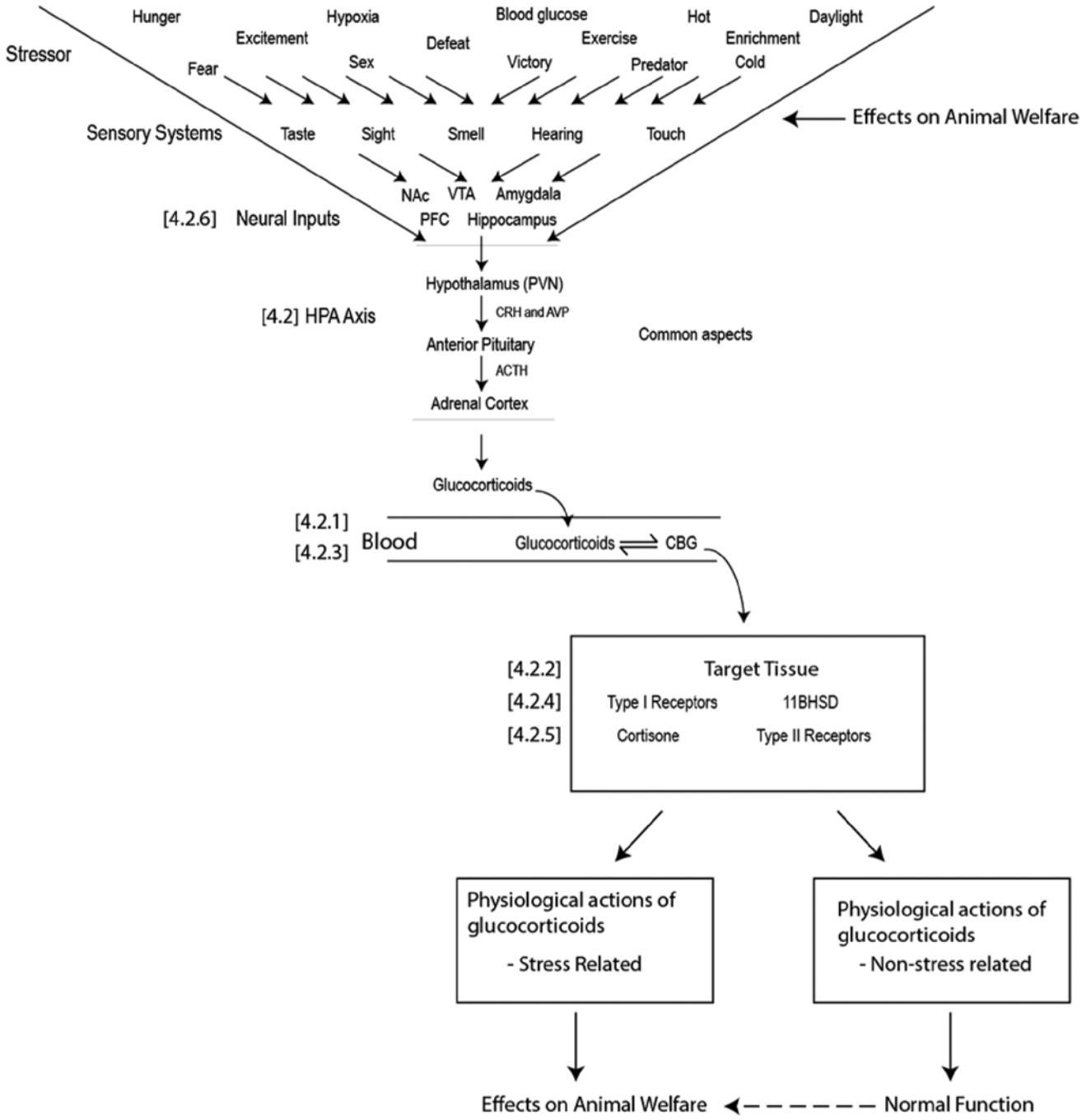

Figure 1. A representation of the physiological systems involved in stress responses and animal welfare. The figure shows the myriad of sensory and neuronal inputs required to detect a stressor and activate the hypothalamo–pituitary–adrenal (HPA) axis together with the aspects of glucocorticoid physiology that can be affected by increased glucocorticoid synthesis. The figure shows that activation of the HPA axis is common to all stress responses whereas the efferent and afferent systems that initiate this activation are unique. Understanding the operation of these systems is potentially informative when assessing animal welfare. In addition, the figure highlights that the effects of stress responses on animal welfare are determined by the neuronal inputs and the consequences of the actions of glucocorticoids. There are stress-related consequences that may have the potential to affect the welfare of an animal. There are also nonstress actions of glucocorticoids that are part of normal functioning. Only when non-stress-related actions become dysfunction, such as when prolonged or excessive, will they possibly impact animal welfare. This is indicated by the dashed arrow. Square brackets indicate the corresponding section in the text. NAc = nucleus accumbens; VTA = ventral tegmental area; PFC = prefrontal cortex; PVN = paraventricular nucleus; CRH = corticotropin releasing hormone; AVP = arginine vasopressin; CBG = corticosteroid-binding globulin; 11βHSD = 11 β-hydroxysteroid dehydrogenase.

quired, thereby increasing the intracellular concentration of cortisol without activating the HPA axis. Conversely, 11 β-HSD 2 acts as a buffer to cortisol action by converting active cortisol to inactive cortisone, thus protecting tissue from glucocorticoid actions.

A dysfunction in the 11 β-HSD system has been associated with a number of metabolic disorders and has been shown to elevate (or reduce) the amount of glucocorticoid available within target tissue (Seckl and Walker, 2001; Andrews et al., 2002; Chapman et

al., 2013). Investigations into the 11 β-HSD system have shown that the tissue concentration of glucocorticoids can be higher than the plasma concentration of glucocorticoids (Andrews et al., 2002; Tomlinson et al., 2004; Cohen et al., 2009, 2012; Vassiliadi et al., 2013). Tissue cortisol obtained via subcutaneous microdialysis of adipose tissue from humans does not always correlate with plasma cortisol concentrations (Cohen et al., 2009, 2012; Vassiliadi et al., 2013), and studies in patients with sepsis have shown that interstitial and tissue cortisol concentrations can increase independent of an increase in plasma cortisol (Vassiliadi et al., 2013). Likewise, in patients with glucose intolerance, tissue cortisol levels can be higher than those in the plasma and these changes at the tissue level can occur in the absence of a change in plasma cortisol (Andrews et al., 2002). In this instance, tissue cortisol proved more useful than plasma cortisol when evaluating stress responses in these patients (Andrews et al., 2002). This increase has been attributed to an increase in 11 β-HSD 1 activity and can occur without stimulation of the HPA axis and without an increase in plasma glucocorticoid.

Conversely, tissue can be protected from the effects of increased plasma glucocorticoid concentrations by the actions of 11 β-HSD 2 (Morgan et al., 2014). When the adrenal glands have been activated to synthesize glucocorticoids, the 11 β-HSD 2 system can be upregulated to convert cortisol to cortisone. Effectively, this protects the target tissue from the actions of glucocorticoids and provides a pool of cortisone that can be used by metabolic tissues after conversion to cortisol by 11 β-HSD 1 (Tomlinson et al., 2004). Therefore, 11 β-HSD 1 and 2 are tissue-specific regulators of glucocorticoid actions and stress responses (Tomlinson et al., 2004; McNeil et al., 2007; Morgan et al., 2014).

It is apparent from this forgoing discussion that the 11 β-HSD system is essential in determining the physiological outcomes from the actions of glucocorticoids in target tissues. A corollary of this is that an understanding of the 11 β-HSD system may be necessary to interpret whether specific actions of glucocorticoids will impact the welfare of an animal (Fig. 1). This understanding is unlikely to be obtained from serum or plasma measurements of glucocorticoids alone.

Tissue Glucocorticoids: Where Glucocorticoids Act

There is a pool of glucocorticoids within the cells of target tissue that will not be evident from measuring glucocorticoids in blood. Breuner et al. (2013) suggested that a full understanding of stress responses requires inclusion of a suite of downstream measures and not just a focus on the blood (Breuner et al., 2013). An alternate approach to evaluating stress could focus on the site of glucocorticoid action within the target tissues (Cohen and Venkatesh, 2009; Cohen et al., 2009, 2012; Ralph et al., 2015). To this end, the tissue concentration of glucocorticoids has been evaluated. The tissue concentration of glucocorticoids is considered to be the intracellular and interstitial concentration because this reflects the glucocorticoid pool available to bind to the glucocorticoid receptors (Cohen and Venkatesh, 2009; Cohen et al., 2012; Perogamvros et al., 2012). Estrada-Y-Martin and Orlander (2011) suggested that tissue glucocorticoid levels mimic free glucocorticoid levels in the plasma and that both are approximately 10% of plasma total glucocorticoid. Therefore, tissue glucocorticoid levels could match the level and timing of the free plasma glucocorticoid. Conversely, Breuner et al. (2013) proposed that far more than the 10% of glucocorticoid that is free in the bloodstream during stress will make it into tissues and activate receptors. They contended that tissue glucocorticoids will increase over a different timecourse than plasma glucocorticoids with the amount of glucocorticoids that make it into tissue depending on the amount of corticosteroid-binding globulin present in the plasma relative to the level of glucocorticoid degradation in the liver (Breuner et al., 2013). Nonetheless, these authors conceded that their argument is a hypothesis that needs to be experimentally tested. There are a number of mechanisms that can modulate tissue glucocorticoid availability. There is agreement in the literature that understanding these mechanisms is essential to understanding stress responses and that further research in this area is needed (Cohen and Venkatesh, 2009; Llompart-Pou et al., 2010; Odermatt and Nashev, 2010; Perogamvros et al., 2012; Breuner et al., 2013; Vassiliadi et al., 2013). This research needs to extend the understanding to the assessment of animal welfare (Fig. 1).

There is substantial evidence indicating that extending our investigations of stress responses to the target tissue may prove useful when evaluating animal welfare. Investigations within the target tissue may provide information about the actions of glucocorticoids and, therefore, the physiological and behavioral consequences of these actions (Cohen and Venkatesh, 2009; Cohen et al., 2009, 2012; Perogamvros et al., 2012; Breuner et al., 2013; Chapman et al., 2013; Vassiliadi et al., 2013; Ralph et al., 2015). It is the consequences of the actions of glucocorticoids that may impact welfare, and these can rarely be determined by peripheral measures alone. For example, Ralph et al. (2015) showed that increased corticosterone in laying hens evoked an increase in gluconeogenesis only when corticosterone in the liver was increased and glucose was depleted. Therefore, a change in plasma

corticosterone was not reflective of the effect of the stressor on the welfare of the hens.

THE NEUROCIRCUITRY INVOLVED IN ACTIVATION OF THE HYPOTHALAMO–PITUITARY–ADRENAL AXIS

The brain responds to stress in a complex, orchestrated fashion and this response requires activation of structures involved in sensory, motor, autonomic, cognitive, and emotional functions (López et al., 1999). Neurons afferent to the hypothalamus receive and process information from external and internal cues that is relayed to the paraventricular nucleus. Multiple brain regions are involved in stress responses before the paraventricular nucleus is affected and these include brain regions that control fear, reward, and learning as well as brain regions that control homeostatic set points such as blood pressure and glucose concentration. These brain regions are activated in a stressor-specific manner and this is dependent on the attributes of the stressor (Herman and Cullinan, 1997). In rodents, stressors such as restraint, foot shock, or exposure to a novel environment activate the prefrontal cortex, hippocampus, or amygdala whereas stressors such as hypoxia and hypoglycemia do not activate these higher brain regions and tend to act directly on the paraventricular nucleus (Herman and Cullinan, 1997). This indicates that some stressors require processing in higher brain regions whereas others do not. Knowledge of this afferent neurocircuitry may enable one to determine which type of stressor initiated activation of the HPA axis. For example, this is vital when attempting to determine if an increase in glucocorticoids is evidence of arousal and reward or evidence of fear and anxiety. Measures of glucocorticoids in the blood will not necessarily provide information about the brain regions that were involved in relaying information to the paraventricular nucleus. Nevertheless, research in a range of species, especially sheep, has shown that specific stressors will yield particular ranges of concentrations of glucocorticoids in blood (Turner et al., 2012), and this is somewhat useful when interpreting the impact of stressors on the physiology and behavior of an animal. Although the usefulness of such measures for assessing welfare would appear to be most applicable to biological functioning, it is still limited by not knowing the source of the neural information relayed to the paraventricular nucleus. Clearly, there is a need to understand the neurocircuitry involved in stress responses. Indeed, not only will this assist in determining biological functioning but also it will open the way to understanding affective states.

STRESS AND ANIMAL WELFARE

The measurement of glucocorticoids in animal welfare science is used to identify stress responses, and it is common to associate increased stress with compromised welfare. Nonetheless, stress responses are designed to be adaptive and for the most part in a healthy animal they are. We recently reviewed stress responses and health in humans and a general notion was that short-term stress responses, normally referred to as acute responses, are mostly positive and adaptive whereas when stress systems are activated repeatedly or continuously over long periods the effects can be deleterious for health (Turner et al., 2012). The same view could be applied to using stress responses to assist in the assessment of the welfare of animals but, as indicated above, the difficulty comes from being unable to interpret the welfare state of an animal from peripheral measures of hormones such as glucocorticoids. These measures provide no evidence of the original stimulus that activated the paraventricular nucleus. For example, by measuring an increase in plasma glucocorticoids it is not possible to determine whether the paraventricular nucleus was activated by fear, reward, or hypoglycemia. Moreover, the HPA axis can be stimulated by both seemingly positive conditions, such as winning a social interaction, and negative conditions, such as losing a social interaction (Koolhaas et al., 1999). Furthermore, repeatedly chronically raised peripheral concentrations of glucocorticoids do not inevitably mean compromised welfare and acute increases do not automatically signal a state of normality. Importantly, the peripheral measures of glucocorticoids may not edify the impact of the environment on the biological functioning and affective state of the animal.

Just as repeated or extended continuous activation of the HPA axis can be deleterious for human health (Turner et al., 2012), so it can also be for the health of animals and, in turn, their welfare. Not surprisingly, there is a common belief that so-called chronic activation of the HPA axis will negatively impact physiological and behavioral functioning (Barnett, 1987). Conversely, there is a general belief that acute stress responses are unlikely to have detrimental impacts on animal health and welfare, although this may not always hold true, especially if the response occurs at a critical time. For example, Moberg (1987b,a) championed the hypothesis that reproduction in females may be impaired if the HPA axis is acutely activated at a critical time during the follicular phase. It is feasible that this premise may also apply to other physiological and behavioral systems that could influence the welfare of an animal. With respect to reproduction, we did not find inhibitory effects of acute increases in cortisol on reproduction in female pigs (Turner et al., 1998, 1999) but we showed in ewes that

acute increases in cortisol at critical times impaired both the surge in luteinizing hormone required for ovulation (Pierce et al., 2009; Wagenmaker et al., 2009) and sexual behavior (Pierce et al., 2008; Papargiris et al., 2011). The differences between studies may be related to species or to the magnitude and/or timing of the presence of high concentrations of glucocorticoids. Nevertheless, it is apparent that, under some circumstances, acute excursions in the circulating concentrations of glucocorticoids can impair physiological and behavioral function. Indeed, this has been shown in humans, where the timing of an acute stress response may result in ischemic heart injury and failure (Sapolsky, 2000).

Even though there seems to be reasonable evidence that chronic activation of the HPA axis can compromise the welfare of animals, the extent to which measures of glucocorticoids can assist in understanding the impact on biological functioning or affective states is debatable. What levels of glucocorticoids in the blood indicate that welfare will be detrimentally affected? That chronic stress can impair the welfare of animals has been eloquently shown in studies with pigs (Barnett et al., 1984, 1985). Nonetheless, work is needed before measures of glucocorticoids in blood can be used to help assess animal welfare.

At least part of the approach to being able to use measures of glucocorticoids in the assessment of animal welfare lies in understanding the activity of the HPA axis in response to specific stressors. This has been studied in detail in sheep. Turner et al. (2010) characterized the normal cortisol response of sheep to a range of stressors. They were able to describe the normal response of sheep to exercise, isolation and restraint stress, immune challenge with an endotoxin, and wetting stress. This work showed that in response to exercise plasma, cortisol increased to 60 to 70 ng/mL; in response to isolation and restraint stress, plasma cortisol increased to 30 to 40 ng/mL; and in response to endotoxin, plasma cortisol increased to 110 to 130 ng/mL (Turner et al., 2010). Similar research in sheep has shown that in response to audiovisual stress that included a barking dog, plasma cortisol increased to 80 to 100 ng/mL, and in response to insulin, plasma cortisol increased to 60 to 80 ng/mL (Tilbrook et al., 2006; Turner et al., 1999, 2002, 2012). Research in pigs has also given insight into HPA axis responses to stress. Confinement of a female pig for 60 min induced a plasma cortisol concentration of 94 ng/mL, snout roping for 5 min induced a peak plasma cortisol concentration of 108 ng/mL, mating induced a peak of greater than 60 ng/mL, and introduction of a sow to a boar induced 100 ng/mL (Turner et al., 2002). Collectively, these studies show that there are a range of normal or appropriate cortisol responses to stressors. To be able to use this information to assist in the assessment of the welfare of an animal, there is an absolute requirement for research that determines the impacts of these varying responses on the physiology and behavior of the animal. There is a critical gap in knowledge in this regard. Therefore, in addition to an appreciation of the neurophysiology associated with various stressors and the fate and actions of glucocorticoids at the cellular level, we argue that, with appropriate research, it may be possible to develop a diagnostic paradigm of glucocorticoid measures that can be used in the assessment of animal welfare.

GLUCOCORTICOID MEASURES AND THE ANIMAL WELFARE FRAMEWORKS

The Biological Functioning Framework

Given that the biological functioning framework concerns a range of behavioral and physiological responses that involve all bodily functions, an understanding of the actions of glucocorticoids, and the consequences of these actions, is critical to assess the welfare of an animal. As mentioned, this will unlikely be possible from measures of circulating glucocorticoids, although such measures are useful in establishing that the HPA axis has been activated. Essentially, a complete understanding of the stimuli that induce activity of the HPA axis; the transport, protection, liberation, and metabolism of glucocorticoids; the availability of type I and type II glucocorticoids receptors; and the molecular outcomes of receptor binding are necessary to assess the role of glucocorticoids in biological functioning (Fig. 1).

The Affective States Framework

Affect is the hedonic quality of pleasure and displeasure and, as indicated earlier, evaluation of affective states, both positive and negative, is one approach to assessing animal welfare (Berridge and Kringelbach, 2013; Hemsworth et al., 2015). Positive and negative affective experiences evoke glucocorticoid synthesis, making assessment of animal welfare under this framework virtually impossible from measuring circulating glucocorticoids. Although it is challenging to understand affective states from measuring these hormones, extensive research has elucidated the neural inputs that influence the activity of the HPA axis during positive and negative affective states. This neurocircuitry could be examined to better understand animal welfare.

Positive Affective States

Positive affective states such as arousal, excitement, anticipation, and reward can evoke increased synthesis of catecholamines and glucocorticoids. The reward mechanism comprises the prefrontal cortex, ventral tegmental area, and the nucleus accumbens (Alcaro et al., 2007). The prefrontal cortex integrates sensory and motor information essential to goal-directed behavior and, through dopaminergic projections to the ventral tegmental area and nucleus accumbens, controls reward (Mora et al., 2012). Collectively, these brain regions are referred to as the mesolimbic dopaminergic system and are the brain regions activated when reward evokes glucocorticoid synthesis (Kelley and Berridge, 2002). The interaction between the paraventricular nucleus and the mesolimbic dopamine system is bidirectional in that stress can activate the mesolimbic dopaminergic system and rewarding experiences can evoke corticotropin-releasing hormone synthesis in the paraventricular nucleus. In humans, the mesolimbic dopamine system has been implicated in diseases such as schizophrenia, depression, anxiety, attention deficit hyperactivity disorder, and drug addiction (Alcaro et al., 2007), although disease states such as this have not been studied from the perspective of animal welfare. These brain regions are activated during rewarding experiences such as consensual sex and after administration of cocaine and amphetamine (Alcaro et al., 2007). Dopamine release in the nucleus accumbens increases during stressful situations and the glucocorticoid response to stressors is altered when one or all of these regions is lesioned (Mora et al., 2012). Rodents prefer corticosterone-laced water over distilled water and will intravenously or orally self-administer corticosterone (Piazza et al., 1991; Deroche et al., 1992). This causes an increase in dopamine in the nucleus accumbens and indicates that there is a rewarding and reinforcing aspect to corticosterone (Graf et al., 2013). Moreover, molecular and morphological changes have been recorded in these brain regions when animals are housed in enriched environments (Fox et al., 2006; Segovia et al., 2009). These changes result in enhanced learning and memory and indeed enhanced resilience or ability to cope with stressors (Lyons et al., 2010).

Therefore, increased glucocorticoid synthesis is, at times, evidence of a positive affective experience because rewarding experiences evoke glucocorticoid synthesis. This questions the use of glucocorticoids as an indicator of compromised welfare and suggests that, in keeping with glucoregulatory role of glucocorticoids, increased synthesis indicates anticipation of increased energetic needs. Moreover, it is important to recognize that increased glucocorticoid synthesis could equally be interpreted as indicating improved welfare, based on the knowledge that reward evokes glucocorticoid synthesis. This supports the thesis that it is the consequences of the actions of glucocorticoids, not the glucocorticoids per se, that are important to welfare.

Negative Affective States

Negative affective states such as fear, anxiety, and pain can evoke increased HPA axis activity similarly to that induced by positive affective states. Again, an examination of the afferent neurocircuitry that regulates these states is required to assess affective states and, in turn, assess animal welfare.

The brain regions involved in eliciting HPA axis responses to fear are the medial prefrontal cortex, the amygdala, and the periaqueductal gray (Chan et al., 2011). The medial prefrontal cortex is believed to regulate fear behavior by modulating the amygdala and the periaqueductal gray (Chan et al., 2011). Two subregions of the medial prefrontal cortex are believed to play opposing roles in fear behavior (Chan et al., 2011). The prelimbic subdivision promotes the expression of fear by increasing amygdala output, whereas the infralimbic subdivision inhibits the expression of fear behavior by decreasing amygdala activity. The medial prefrontal cortex has direct projections to the periaqueductal gray and regulates defensive behavior (Davis, 1997). The amygdala is essential for proper expression of fear behaviors and, in particular, conditioned fear (Kolber et al., 2008). The amygdala has direct neural projections to the lateral hypothalamus that appear to be involved in activation of the sympathetic autonomic nervous system during fear (Davis, 1997). In addition, there are direct projections from the central nucleus of the amygdala to the paraventricular nucleus and indirect projections to the paraventricular nucleus via the stria terminalis and preoptic area (Davis, 1997). These projections likely mediate the increase in glucocorticoids seen during fear behaviors whereas electrical stimulation of the amygdala has been shown to increase corticosterone release in rats (Mason, 1959; Dunn and Whitener, 1986). In addition, corticotropin-releasing hormone is increased in the central amygdala after conditioned fear and when corticosterone is administered to the central amygdala (Shepard et al., 2000; Roozendaal et al., 2002).

Extensive knowledge of the neuroanatomy and neurophysiology of reward and fear (as well as the neuroanatomy and neurophysiology of other emotional states) exists, and this serves to demonstrate that an increase in glucocorticoids can be evidence of a positive affective state or a negative affective state. From the description above, one can appreciate that

circulating glucocorticoids offer little understanding of the welfare of an animal under the affective states framework because fear and reward both initiate glucocorticoid synthesis. Nonetheless, it does demonstrate that when neuroscience is incorporated into the biological functioning framework, this can inform the affective states framework and result in an improved understanding of animal welfare (Fig. 1).

CONCLUSION

Glucocorticoids are commonly measured during studies of stress, which makes sense, but the usefulness of these measures when understanding stress responses and how these relate to the assessment of animal welfare is not always clear. Despite this, measures of glucocorticoids in animal welfare science are commonplace. An increase in plasma glucocorticoids can equally indicate reward or fear; likewise, greater plasma glucocorticoid concentrations can increase fitness, decrease fitness, or have little effect on fitness. Activation of the HPA axis does not necessarily behest negative consequences for the welfare of an animal. Furthermore, measuring glucocorticoids in blood (and other media such as saliva) provides information about HPA axis activation but little else in terms of effect on physiological and behavioral functioning. Therefore, whether using the biological functioning or affective states frameworks, or both, for the assessment of animal welfare, it is important to consider why the HPA axis has been activated and the physiological consequences of that activation. This means understanding the sensory systems involved in detecting stressors, the central inputs that stimulate the HPA axis, and the metabolic fate and actions of glucocorticoids within target tissues. It is these aspects of physiology that have the potential to impact the welfare of an animal, and indeed, it is these aspects of physiology that have the potential to enlighten the animal welfare debate. Developing an intimate understanding of the neurophysiology that facilitates HPA axis activation and of glucocorticoid physiology that affects change in target tissue will reduce the ambiguity around measuring glucocorticoids in the assessment of animal welfare. Research in these areas will expand our understanding of animal welfare and develop advanced animal welfare assessment techniques. Altogether, this approach will enable a more comprehensive assessment of the welfare of animals.

LITERATURE CITED

Alcaro, A., R. Huber, and J. Panksepp. 2007. Behavioral functions of the mesolimbic dopaminergic system: An affective neuroethological perspective. Brain Res. Brain Res. Rev. 56:283–321. doi:10.1016/j.brainresrev.2007.07.014.

Alexander, S. L., and C. H. G. Irvine. 1998. The effect of social stress on adrenal axis activity in horses: The importance of monitoring corticosteroid-binding globulin capacity. J. Endocrinol. 157:425–432. doi:10.1677/joe.0.1570425.

Andrews, R. C., O. Herlihy, D. E. W. Livingstone, R. Andrew, and B. R. Walker. 2002. Abnormal cortisol metabolism and tissue sensitivity to cortisol in patients with glucose intolerance. J. Clin. Endocrinol. Metab. 87:5587–5593. doi:10.1210/jc.2002-020048.

Andrews, R. C., and B. R. Walker. 1999. Glucocorticoids and insulin resistance: Old hormones, new targets. Clin. Sci. 96:513–523. doi:10.1042/cs0960513.

Bamberger, C. M., H. M. Schulte, and G. P. Chrousos. 1996. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. Endocr. Rev. 17:245–261. doi:10.1210/edrv-17-3-245.

Barnett, J. L. 1987. The physiological concept of stress is useful for assessing welfare. Aust. Vet. J. 64:195–196. doi:10.1111/j.1751-0813.1987.tb09686.x.

Barnett, J. L., G. M. Cronin, and C. G. Winfield. 1981. The effects of individual and group penning of pigs on total and free plasma corticosteroids and the maximum corticosteroid binding capacity. Gen. Comp. Endocrinol. 44:219–225. doi:10.1016/0016-6480(81)90251-3.

Barnett, J. L., G. M. Cronin, C. G. Winfield, and A. M. Dewar. 1984. The welfare of adult pigs: The effects of five housing treatments on behaviour, plasma corticosteroids and injuries. Appl. Anim. Behav. Sci. 12:209–232. doi:10.1016/0168-1591(84)90115-1.

Barnett, J. L., C. G. Winfield, G. M. Cronin, P. H. Hemsworth, and A. M. Dewar. 1985. The effect of individual and group housing on behavioural and physiological responses related to the welfare of pregnant pigs. Appl. Anim. Behav. Sci. 14:149–161. doi:10.1016/0168-1591(85)90026-7.

Berridge, K. C., and M. L. Kringelbach. 2013. Neuroscience of affect: Brain mechanisms of pleasure and displeasure. Curr. Opin. Neurobiol. 23:294–303. doi:10.1016/j.conb.2013.01.017.

Brambell, F. W. R., D. S. Barbour, M. B. Barnett, T. K. Ewer, A. Hobson, and H. Pitchforth. S. W. R., W. H. Thorpe, and F. J. W. Winship. 1965. Report of the technical committee to enquire in the welfare of animals kept under intensive husbandry systems. Her Majesty's Stationery Office, London, UK.

Breuner, C. W., B. Delehanty, and R. Boonstra. 2013. Evaluating stress in natural populations of vertebrates: Total CORT is not good enough. Funct. Ecol. 27:24–36. doi:10.1111/1365-2435.12016.

Breuner, C. W., and M. Orchinik. 2002. Plasma binding proteins as mediators of corticosteroid action in vertebrates. J. Endocrinol. 175:99–112. doi:10.1677/joe.0.1750099.

Breuner, C. W., M. Orchinick, T. P. Hahn, S. L. Meddle, I. T. Moore, N. T. Owen-Ashley, T. S. Sperry, and J. S. Wingfield. 2003. Differential mechanisms for regulation of the stress response across latitudinal gradients. Am. J. Physiol. Regul. Integr. Comp. Physiol. 285:R594–R600. doi:10.1152/ajpregu.00748.2002.

Bright, G. M. 1995. Corticosteroid-binding globulin influences kinetic parameters of plasma cortisol transport and clearance. J. Clin. Endocrinol. Metab. 80:770–775.

Cannon, W. 1929. Bodily changes in pain, hunger, fear, and rage. Appleton, New York, NY.

Cannon, W. 1932. The wisdom of the body. Norton, New York, NY.

Cawadias, A. P. 1940. The history of endocrinology. Proc. R. Soc. Med. 34:303–307.

Chacko, S. K., J. Ordonez, P. J. J. Sauer, and A. L. Sunehag. 2011. Gluconeogenesis is not regulated by either glucose or insulin in extremely low birth weight infants receiving total parenteral nutrition. J. Pediatr. 158:891–896. doi:10.1016/j.jpeds.2010.12.040.

Chan, T., K. Kyere, B. R. Davis, A. Shemyakin, P. A. Kabitzke, H. N. Shair, G. A. Barr, and C. P. Wiedenmayer. 2011. The role of the medial prefrontal cortex in innate fear regulation in infants, juveniles, and adolescents. J. Neurosci. 31:4991–4999. doi:10.1523/JNEUROSCI.5216-10.2011.

Chapman, K., M. Holmes, and J. Seckl. 2013. 11β-hydroxysteroid dehydrogenases intracellular gate-keepers of tissue glucocorticoid action. Physiol. Rev. 93:1139–1206. doi:10.1152/physrev.00020.2012.

Chaves, V. E. 2006. Glyceroneogenesis is reduced and glucose uptake is increased in adipose tissue from cafeteria diet-fed rats independently of tissue sympathetic innervation. J. Nutr. 136:2475–2480.

Chrousos, G. P., and T. Kino. 2005. Intracellular glucocorticoid signaling: A formerly simple system turns stochastic. Sci. STKE 2005:pe 48.

Cohen, J., R. Deans, A. Dalley, J. Lipman, M. S. Roberts, and B. Venkatesh. 2009. Measurement of tissue cortisol levels in patients with severe burns: A preliminary investigation. Crit. Care 13:R189.

Cohen, J., M. L. Smith, R. V. Deans, C. J. Pretorius, J. P. J. Ungerer, T. Tan, M. Jones, and B. Venkatesh. 2012. Serial changes in plasma total cortisol, Plasma free cortisol, and tissue cortisol activity in patients with septic shock: An observational study. Shock 37:28–33. doi:10.1097/SHK.0b013e318239b809.

Cohen, J., and B. Venkatesh. 2009. Assessment of tissue cortisol activity. Crit. Care Resusc. 11:287–289.

Dallman, M. F., A. M. Strack, S. F. Akana, M. J. Bradbury, E. S. Hanson, K. A. Scribner, and M. Smith. 1993. Feast and famine: Critical role of glucocorticoids with insulin in daily energy flow. Front. Neuroendocrinol. 14:303–347. doi:10.1006/frne.1993.1010.

Davis, M. 1997. Neurobiology of fear responses: The role of the amygdala. J. Neuropsychiatry Clin. Neurosci. 9:382–402. doi:10.1176/jnp.9.3.382.

De Kloet, E. R. 2004. Hormones and the stressed brain. Ann. N. Y. Acad. Sci. 1018:1–15. doi:10.1196/annals.1296.001.

Delitala, G., P. J. Trainer, O. Oliva, G. Fanciulli, and A. B. Grossman. 1994. Opioid peptide and α-adrenoceptor pathways in the regulation of the pituitary-adrenal axis in man. J. Endocrinol. 141:163–168. doi:10.1677/joe.0.1410163.

Deroche, V., P. V. Piazza, P. Casolini, S. Maccari, M. Le Moal, and H. Simon. 1992. Stress-induced sensitization to amphetamine and morphine psychomotor effects depend on stress-induced corticosterone secretion. Brain Res. 598:343–348. doi:10.1016/0006-8993(92)90205-N.

Devenport, L., A. Knehans, A. Sundstrom, and T. Thomas. 1989. Corticosterone's dual metabolic actions. Life Sci. 45:1389–1396. doi:10.1016/0024-3205(89)90026-X.

Dunn, J. D., and J. Whitener. 1986. Plasma corticosterone responses to electrical stimulation of the amygdaloid complex: Cytoarchitectural specificity. Neuroendocrinology 42:211–217. doi:10.1159/000124442.

Echternkamp, S. E. 1984. Relationship between LH and cortisol in acutely stressed beef cows. Theriogenology 22:305–311. doi:10.1016/0093-691X(84)90487-4.

Estrada-Y-Martin, R. M., and P. R. Orlander. 2011. Salivary cortisol can replace free serum cortisol measurements in patients with septic shock. Chest. 140 1216-1222.

Feldman, J. M., J. W. Plonk, and C. H. Bivens. 1975. The role of cortisol and growth hormone in the counter regulation of insulin induced hypoglycemia. Horm. Metab. Res. 7:378–381. doi:10.1055/s-0028-1093731.

Follenius, M., C. Simon, G. Brandenberger, and P. Lenzi. 1987. Ultradian plasma corticotropin and cortisol rhythms: Time-series analyses. J. Endocrinol. Invest. 10:261–266. doi:10.1007/BF03348128.

Fowden, A. L., J. Mijovic, and M. Silver. 1993. The effects of cortisol on hepatic and renal gluconeogenic enzyme activities in the sheep fetus during late gestation. J. Endocrinol. 137:213–222. doi:10.1677/joe.0.1370213.

Fox, C., Z. Merali, and C. Harrison. 2006. Therapeutic and protective effect of environmental enrichment against psychogenic and neurogenic stress. Behav. Brain Res. 175:1–8. doi:10.1016/j.bbr.2006.08.016.

Franko, K. L., A. J. Forhead, and A. L. Fowden. 2010. Differential effects of prenatal stress and glucocorticoid administration on postnatal growth and glucose metabolism in rats. J. Endocrinol. 204:319–329. doi:10.1677/JOE-09-0390.

Fulkerson, W. J., and B. Y. Tang. 1979. Ultradian and circadian rhythms in the plasma concentration of cortisol in sheep. J. Endocrinol. 81:135–141. doi:10.1677/joe.0.0810135.

Goldstein, R. E., L. Rossetti, B. A. J. Palmer, R. Liu, D. Massillon, M. Scott, D. Neal, P. Williams, B. Peeler, and A. D. Cherrington. 2002. Effects of fasting and glucocorticoids on hepatic gluconeogenesis assessed using two independent methods in vivo. Am. J. Physiol. Endocrinol. Metab. 283:E946–E957. doi:10.1152/ajpendo.00320.2002.

Graf, E. N., R. A. Wheeler, D. A. Baker, A. L. Ebben, J. E. Hill, J. R. McReynolds, M. A. Robble, O. Vranjkovic, D. S. Wheeler, J. R. Mantsch, and P. J. Gasser. 2013. Corticosterone acts in the nucleus accumbens to enhance dopamine signaling and potentiate reinstatement of cocaine seeking. J. Neurosci. 33:11800–11810. doi:10.1523/JNEUROSCI.1969-13.2013.

Hemsworth, P. H., D. J. Mellor, G. M. Cronin, and A. J. Tilbrook. 2015. Scientific assessment of animal welfare. N. Z. Vet. J. 63:24–30. doi:10.1080/00480169.2014.966167.

Herman, J. P., and W. E. Cullinan. 1997. Neurocircuitry of stress: Central control of the hypothalamo–pituitary–adrenocortical axis. Trends Neurosci. 20:78–84. doi:10.1016/S0166-2236(96)10069-2.

Johnson, E. O., T. C. Kamilaris, G. P. Chrousos, and P. W. Gold. 1992. Mechanisms of stress: A dynamic overview of hormonal and behavioral homeostasis. Neurosci. Biobehav. Rev. 16:115–130. doi:10.1016/S0149-7634(05)80175-7.

Kelley, A. E., and K. C. Berridge. 2002. The neuroscience of natural rewards: Relevance to addictive drugs. J. Neurosci. 22:3306–3311.

Kino, T. 2007. Tissue glucocorticoid sensitivity: beyond stochastic regulation on the diverse actions of glucocorticoids. Horm. Metab. Res. 39:420–424. doi:10.1055/s-2007-980193.

Kolber, B. J., M. S. Roberts, M. P. Howell, D. F. Wozniak, M. S. Sands, and L. J. Muglia. 2008. Central amygdala glucocorticoid receptor action promotes fear-associated CRH activation and conditioning. Proc. Natl. Acad. Sci. USA 105:12004–12009. doi:10.1073/pnas.0803216105.

Koolhaas, J. M., S. M. Korte, S. F. De Boer, B. J. Van Der Vegt, C. G. Van Reenen, H. Hopster, I. C. De Jong, M. A. W. Ruis, and H. J. Blokhuis. 1999. Coping styles in animals: Current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23:925–935. doi:10.1016/S0149-7634(99)00026-3.

Kyrou, I., and C. Tsigos. 2009. Stress hormones: Physiological stress and regulation of metabolism. Curr. Opin. Pharmacol. 9:787–793. doi:10.1016/j.coph.2009.08.007.

Le, P. P., J. R. Friedman, J. Schug, J. E. Brestelli, J. B. Parker, I. M. Bochkis, and K. H. Kaestner. 2005. Glucocorticoid receptor-dependent gene regulatory networks. PLoS Genet. 1:0159–0170.

Lim-Tio, S. S., and P. J. Fuller. 1998. Intracellular signaling pathways confer specificity of transactivation by mineralocorticoid and glucocorticoid receptors. Endocrinology 139:1653–1661.

Lim-Tio, S. S., M.-C. Keightley, and P. J. Fuller. 1997. Determinants of specificity of transactivation by the mineralocorticoid or glucocorticoid receptor. Endocrinology 138:2537–2543.

Llompart-Pou, J. A., G. Pérez, J. Pérez-Bárcena, M. Brell, J. Ibáñez, M. Riesco, J. M. Abadal, J. Homar, P. Marsé, J. Ibáñez, B. Burguera, and J. M. Raurich. 2010. Correlation between brain interstitial and total serum cortisol levels in traumatic brain injury. A preliminary study. J. Endocrinol. Invest. 33:368–372. doi:10.1007/BF03346605.

López, J. F., H. Akil, and S. J. Watson. 1999. Neural circuits mediating stress. Biol. Psychiatry 46:1461–1471. doi:10.1016/S0006-3223(99)00266-8.

Lyons, D. M., K. J. Parker, and A. F. Schatzberg. 2010. Animal models of early life stress: Implications for understanding resilience. Dev. Psychobiol. 52:402–410. doi:10.1002/dev.20429.

Maciel, S. M., C. S. Chamberlain, R. P. Wettemann, and L. J. Spicer. 2001. Dexamethasone influences endocrine and ovarian function in dairy cattle. J. Dairy Sci. 84:1998–2009. doi:10.3168/jds.S0022-0302(01)74643-7.

Mason, J. W. 1959. Plasma 17-hydroxycorticosteroid levels during electrical stimulation of the amygdaloid complex in conscious monkeys. Am. J. Physiol. 196:44–48.

McEwen, B. S., and J. C. Wingfield. 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43:2–15. doi:10.1016/S0018-506X(02)00024-7.

McNeil, C. J., M. O. Nwagwu, A. M. Finch, K. R. Page, A. Thain, H. J. McArdle, and C. J. Ashworth. 2007. Glucocorticoid exposure and tissue gene expression of 11β HSD-1 11B HSD-2, and glucocorticoid receptor in a porcine model of differential fetal growth. Reproduction 133:653–661. doi:10.1530/rep.1.01198.

Moberg, G. P. 1987a. Influence of the adrenal axis upon the gonads. Oxf. Rev. Reprod. Biol. 9:456–496.

Moberg, G. P. 1987b. Problems in defining stress and distress in animals. J. Am. Vet. Med. Assoc. 191:1207–1211.

Mora, F., G. Segovia, A. Del Arco, M. De Blas, and P. Garrido. 2012. Stress, neurotransmitters, corticosterone and body-brain integration. Brain Res. 1476:71–85. doi:10.1016/j.brainres.2011.12.049.

Morgan, S. A., E. L. McCabe, L. L. Gathercole, Z. K. Hassan-Smith, D. P. Larner, I. J. Bujalska, P. M. Stewart, J. W. Tomlinson, and G. G. Lavery. 2014. 11β-HSD1 is the major regulator of the tissue-specific effects of circulating glucocorticoid excess. Proc. Natl. Acad. Sci. USA 111:E2482–E2491. doi:10.1073/pnas.1323681111.

Mormède, P., S. Andanson, B. Aupérin, B. Beerda, D. Guémené, J. Malmkvist, X. Manteca, G. Manteuffel, P. Prunet, C. G. van Reenen, S. Richard, and I. Veissier. 2007. Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. Physiol. Behav. 92:317–339. doi:10.1016/j.physbeh.2006.12.003.

Newton, R. 2000. Molecular mechanisms of glucocorticoid action: What is important? Thorax 55:603–613. doi:10.1136/thorax.55.7.603.

Odermatt, A., and L. G. Nashev. 2010. The glucocorticoid-activating enzyme 11[beta]-hydroxysteroid dehydrogenase type 1 has broad substrate specificity: Physiological and toxicological considerations. J. Steroid Biochem. Mol. Biol. 119:1–13. doi:10.1016/j.jsbmb.2010.01.007.

Olsson, T., and R. Sapolsky. 2006. The healthy cortisol response stress in health and disease. In: B. B. Arnetz and R. Ekman, editors, Stress in health and disease. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. p. 214–225.

Papargiris, M. M., E. T. Rivalland, P. H. Hemsworth, A. D. Morrissey, and A. J. Tilbrook. 2011. Acute and chronic stress-like levels of cortisol inhibit the oestradiol stimulus to induce sexual receptivity but have no effect on sexual attractivity or proceptivity in female sheep. Horm. Behav. 60:336–345. doi:10.1016/j.yhbeh.2011.06.008.

Perogamvros, I., D. W. Ray, and P. J. Trainer. 2012. Regulation of cortisol bioavailability – Effects on hormone measurement and action. Nat. Rev. Endocrinol. 8:717–727. doi:10.1038/nrendo.2012.134.

Piazza, P. V., S. Maccari, J. M. Deminière, M. Le Moal, P. Mormède, and H. Simon. 1991. Corticosterone levels determine individual vulnerability to amphetamine self-administration. Proc. Natl. Acad. Sci. USA 88:2088–2092. doi:10.1073/pnas.88.6.2088.

Picard-Hagan, P., V. Gayrard, H. Alvinerie, V. Laroute, C. Touron, O. Andreoletti, and P. L. Toutain. 2000. Naturally occurring scrapie is associated with a lower CBG binding capacity in ewes. J. Endocrinol. 165:527–532. doi:10.1677/joe.0.1650527.

Pierce, B. N., I. J. Clarke, A. I. Turner, E. T. Rivalland, and A. J. Tilbrook. 2009. Cortisol disrupts the ability of estradiol-17beta to induce the LH surge in ovariectomized ewes. Domest. Anim. Endocrinol. 36:202–208. doi:10.1016/j.domaniend.2008.11.003.

Pierce, B. N., P. H. Hemsworth, E. T. Rivalland, E. R. Wagenmaker, A. D. Morrissey, M. M. Papargiris, I. J. Clarke, F. J. Karsch, A. I. Turner, and A. J. Tilbrook. 2008. Psychosocial stress suppresses attractivity, proceptivity and pulsatile LH secretion in the ewe. Horm. Behav. 54:424–434. doi:10.1016/j.yhbeh.2008.04.005.

Qian, X., S. K. Droste, M. Gutièrrez-Mecinas, A. Collins, F. Kersanté, J. M. H. M. Reul, and A. C. E. Linthorst. 2011. A rapid release of corticosteroid-binding globulin from the liver restrains the glucocorticoid hormone response to acute stress. Endocrinology 152:3738–3748. doi:10.1210/en.2011-1008.

Ralph, C. R., P. H. Hemsworth, B. J. Leury, and A. J. Tilbrook. 2015. Relationship between plasma and tissue corticosterone in laying hens (*Gallus gallus domesticus*): Implications for stress physiology and animal welfare. Domest. Anim. Endocrinol. 50:72–82. doi:10.1016/j.domaniend.2014.09.002.

Remage-Healey, L., and L. M. Romero. 2001. Corticosterone and insulin interact to regulate glucose and triglyceride levels during stress in a bird. Am. J. Physiol. Regul. Integr. Comp. Physiol. 281:R994–R1003.

Restitutti, F., M. Raekallio, M. Vainionpää, E. Kuusela, and O. Vainio. 2012. Plasma glucose, insulin, free fatty acids, lactate and cortisol concentrations in dexmedetomidine-sedated dogs with or without MK-467: A peripheral α-2 adrenoceptor antagonist. Vet. J. 193:481–485. doi:10.1016/j.tvjl.2011.12.010.

Romero, L. M., and L. Remage-Healey. 2000. Daily and seasonal variation in response to stress in captive starlings (*Sturnus vulgaris*): Corticosterone. Gen. Comp. Endocrinol. 119:52–59. doi:10.1006/gcen.2000.7491.

Roozendaal, B., K. L. Brunson, B. L. Holloway, J. L. McGaugh, and T. Z. Baram. 2002. Involvement of stress-released corticotropin-releasing hormone in the basolateral amygdala in regulating memory consolidation. Proc. Natl. Acad. Sci. USA 99:13908–13913. doi:10.1073/pnas.212504599.

Sapolsky, R. M. 2000. Stress hormones: Good and bad. Neurobiol. Dis. 7:540–542. doi:10.1006/nbdi.2000.0350.

Sapolsky, R. M., M. L. Romero, and A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21:55–89.

Sawchenko, P. E., H. Y. Li, and A. Ericsson. 1999. Circuits and mechanisms governing hypothalamic responses to stress: A tale of two paradigms. Prog. Brain Res. 122:61–78.

Seckl, J. R., and B. R. Walker. 2001. Minireview: 11-β hydroxysteroid dehydrogenase type 1 – A tissue-specific amplifier of glucocorticoid action. Endocrinology 142:1371–1376.

Segovia, G., A. D. Arco, and F. Mora. 2009. Environmental enrichment, prefrontal cortex, stress, and aging of the brain. J. Neural Transm. 116:1007–1016. doi:10.1007/s00702-009-0214-0.

Selye, H. 1946. The general adaptation syndrome and the diseases of adaptation. J. Clin. Endocrinol. Metab. 6:117–230.

Selye, H. 1955. Stress and disease. Science 122:625–631. doi:10.1126/science.122.3171.625.

Selye, H. 1956. The stress of life. McGraw-Hill, New York, NY.

Shepard, J. D., K. W. Barron, and D. A. Myers. 2000. Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior. Brain Res. 861:288–295. doi:10.1016/S0006-8993(00)02019-9.

Sorrells, S. F., and R. M. Sapolsky. 2007. An inflammatory review of glucocorticoid actions in the CNS. Brain Behav. Immun. 21:259–272. doi:10.1016/j.bbi.2006.11.006.

Tempel, D. L., and S. F. Leibowitz. 1994. Adrenal steroid receptors: Interactions with brain neuropeptide systems in relation to nutrient intake and metabolism. J. Neuroendocrinol. 6:479–501. doi:10.1111/j.1365-2826.1994.tb00611.x.

Thun, R., E. Eggenberger, K. Zerobin, T. Lüscher, and W. Vetter. 1981. Twenty-four-hour secretory pattern of cortisol in the bull: Evidence of episodic secretion and circadian rhythm. Endocrinology 109:2208–2212. doi:10.1210/endo-109-6-2208.

Tilbrook, A. J. 2007. Neuropeptides, stress-related. In: G. Fink, editor, Encyclopedia of stress. Academic Press, Oxford, UK. p. 903–908.

Tilbrook, A. J., and I. J. Clarke. 2006. Neuroendocrine mechanisms of innate states of attenuated responsiveness of the hypothalamo-pituitary adrenal axis to stress. Front. Neuroendocrinol. 27:285–307. doi:10.1016/j.yfrne.2006.06.002.

Tilbrook, A. J., E. A. T. Rivalland, A. I. Turner, G. W. Lambert, and I. J. Clarke. 2008. Responses of the hypothalamopituitary adrenal axis and the sympathoadrenal system to isolation/restraint stress in sheep of different adiposity. Neuroendocrinology 87:193–205. doi:10.1159/000117576.

Tilbrook, A. J., A. I. Turner, M. D. Ibbott, and I. J. Clarke. 2006. Activation of the hypothalamo-pituitary-adrenal axis by isolation and restraint stress during lactation in ewes: Effect of the presence of the lamb and suckling. Endocrinology 147:3501–3509. doi:10.1210/en.2005-1632.

Tomlinson, J. W., E. A. Walker, I. J. Bujalska, N. Draper, G. G. Lavery, M. S. Cooper, M. Hewison, and P. M. Stewart. 2004. 11β-Hydroxysteroid dehydrogenase type 1: A tissue-specific regulator of glucocorticoid response. Endocr. Rev. 25:831–866. doi:10.1210/er.2003-0031.

Turner, A. I., P. H. Hemsworth, B. J. Canny, and A. J. Tilbrook. 1999. Sustained but not repeated acute elevation of cortisol impaired the luteinizing hormone surge, estrus, and ovulation in gilts. Biol. Reprod. 61:614–620. doi:10.1095/biolreprod61.3.614.

Turner, A. I., P. H. Hemsworth, P. E. Hughes, and A. J. Tilbrook. 1998. Repeated acute activation of the hypothalamo-pituitary adrenal axis prior to and during estrus did not affect reproductive performance in gilts. Biol. Reprod. 58:1458–1462. doi:10.1095/biolreprod58.6.1458.

Turner, A. I., P. H. Hemsworth, and A. J. Tilbrook. 2002. Susceptibility of reproduction in female pigs to impairment by stress and the role of the hypothalamo-pituitary-adrenal axis. Reprod. Fertil. Dev. 14:377–391. doi:10.1071/RD02012

Turner, A. I., C. Keating, and A. J. Tilbrook. 2012. Sex differences and the role of sex steroids in sympatho-adrenal medullary system the hypothalamo-pituitary adrenal axis responses to stress. In: S. M. Kahn, editor, Sex steroids. Tech Publishing, Rijeka, Croatia. p. 115–136.

Turner, A. I., E. T. A. Rivalland, I. J. Clarke, and A. J. Tilbrook. 2010. Stressor specificity of sex differences in hypothalamo-pituitary-adrenal axis activity: Cortisol responses to exercise, endotoxin, wetting, and isolation/restraint stress in gonadectomized male and female sheep. Endocrinology 151:4324–4331. doi:10.1210/en.2010-0234.

Vassiliadi, D. A., I. Ilias, M. Tzanela, N. Nikitas, M. Theodorakopoulou, P. Kopterides, N. Maniatis, A. Diamantakis, S. E. Orfanos, I. Perogamvros, A. Armaganidis, B. G. Keevil, S. Tsagarakis, and I. Dimopoulou. 2013. Interstitial cortisol obtained by microdialysis in mechanically ventilated septic patients: Correlations with total and free serum cortisol. J. Crit. Care 28:158–165. doi:10.1016/j.jcrc.2012.07.008.

Wagenmaker, E. R., K. M. Breen, A. E. Oakley, B. N. Pierce, A. J. Tilbrook, A. I. Turner, and F. J. Karsch. 2009. Cortisol interferes with the estradiol-induced surge of luteinizing hormone in the ewe. Biol. Reprod. 80:458–463. doi:10.1095/biolreprod.108.074252.

Wang, M. 2005. The role of glucocorticoid action in the pathophysiology of the metabolic syndrome. Nutr. Metab. 2:3. doi:10.1186/1743-7075-2-3.