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## **The human stress response**

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### **Abstract**

The human stress response has evolved to maintain homeostasis under conditions of real or perceived stress. This objective is achieved through autoregulatory neural and hormonal systems in close association with central and peripheral clocks. The hypothalamic–pituitary–adrenal (HPA) axis is a key regulatory pathway in the maintenance of these homeostatic processes. The end product of this pathway — cortisol — is secreted in a pulsatile pattern, with changes in pulse amplitude creating a circadian pattern. During acute stress, cortisol levels rise and pulsatility is maintained. Although the initial rise in cortisol follows a large surge of adrenocorticotropic hormone (ACTH), if long-term inflammatory stress occurs, ACTH returns to near basal levels while cortisol remains raised as a result of increased adrenal sensitivity. In chronic stress, hypothalamic activation of the pituitary changes from corticotropin-releasing hormone dominant to arginine vasopressin dominant, and cortisol remains raised due at least in part to decreased cortisol metabolism. Acute elevations in cortisol are beneficial to promote survival of the fittest as part of the fight or flight response. However, chronic exposure to stress results in reversal of the beneficial effects with long-term cortisol exposure becoming maladaptive, which can lead to a broad range of problems, including the metabolic syndrome, obesity, cancer, mental health disorders, cardiovascular disease and increased susceptibility to infections. Neuroimmunoendocrine modulation in disease states and glucocorticoid-based therapeutics are also discussed.

## **Key points**

The hypothalamic–pituitary–adrenal (HPA) axis is a key system that synchronises the stress response with circadian regulatory processes.

Regulation of the HPA axis is very dynamic with both ultradian and circadian oscillations.

Short term and longer-term stress result in different regulatory mechanisms involving hypothalamic, pituitary and adrenal activity, as well as cortisol metabolism.

Chronic elevation and non-physiological patterns of cortisol result in poor cognitive, metabolic and immune function.

## **[H1] Introduction**

In response to a stressor the body activates multiple co-ordinated and dynamic processes to restore homeostasis, preserve life and ultimately achieve evolutionary success for the species. The importance of endocrine systems in this homeostatic regulation has been known since the early studies of Hans Selye<sup>1</sup> in the 1930s, when activation of the sympatho–adrenomedullary (SAM) and hypothalamic–pituitary–adrenal (HPA) axes was described in response to physical injury and exertion as well as perceived psychological threats. Interestingly, anticipation of these threats is itself a very potent activator of these systems<sup>2</sup>. Homeostatic processes also interact with internal and external **Zeitgebers** such as the light–dark cycle and internal body clocks<sup>3,4</sup>. These internal clocks enable the body to anticipate regular changes in the environment to ensure optimal fitness across the 24 hours and thus the best chance for survival<sup>5</sup>. The human stress response is an additional homeostatic mechanism that provides a better chance of survival when the body is under threat and mobilises neural and hormonal networks to optimise cognitive, cardiovascular, immunological and metabolic function (figure 1). In this Review we discuss how the HPA axis helps achieve and maintain homeostasis. We will show how it utilises rhythmic 24-hour patterns of secretion to achieve

appropriate tissue activity at different times of the day, and faster ultradian rhythms to optimise tissue specific glucocorticoid signalling and maintain the rapid reactivity necessary for a stress response system.

### **[H1] Circadian clocks**

In the absence of internal or external stressors, the integrity of physiological systems is maintained in a dynamic fashion over 24 h by an internal **circadian clock** that anticipates the changes occurring over the 24-h day. In the past, the neurocentric hierarchical view was that body rhythms were controlled from a master clock in the hypothalamic suprachiasmatic nucleus (SCN)<sup>6</sup>. Now, it is very clear that peripheral clocks also exist in most, if not all, tissues of the body, which have their own autonomous transcriptional autoregulatory feedback loops<sup>7</sup>. The core clock genes, both in the SCN and peripheral clocks, are *CLOCK* and *BMAL1*. Activation of these genes generates a heterodimer of *BMAL1* with either *CLOCK* (or *NPAS2*) which bind at promotor elements called E-boxes to drive genes encoding period 1-3, cryptochrome 1-2 and nuclear receptor subfamily 1-2. The resultant proteins then feedback to repress *BMAL1* by a series of feedback loops generating a 24-hour rhythm<sup>8,9</sup> which allows time of day dependent regulation of downstream genomic pathways. This rhythmicity is crucial for homeostasis. The body can only behave optimally when all biological rhythms are in synchrony. However, with our increasingly chaotic lifestyles this orderly physiological regulation is steadily being disrupted, which can result in chronodisruption<sup>4,10</sup>. This desynchronization between cellular oscillators in the SCN and peripheral tissues can manifest as negative health outcomes in the form of cardiovascular, metabolic, cognitive and immune dysfunction<sup>4,10-14</sup>.

### **[H1] HPA axis and circadian rhythmicity**

The HPA is critical for life and is a major part of our homeostatic regulatory system<sup>15</sup>. The output of this system is the endogenous glucocorticoid corticosterone (in rodents) or cortisol (in humans), which are collectively referred to as CORT. Glucocorticoids have diverse and far reaching effects, which is why they are such successful therapeutic agents; however, this diversity is a double-edged sword and excess levels of glucocorticoids result in a myriad of unwanted adverse effects including diabetes, hypertension, immune dysregulation and osteoporosis<sup>16</sup>. Glucocorticoids exhibit powerful anti-inflammatory functions both at a whole cell and at transcriptional level. They can induce apoptosis of T lymphocytes, neutrophils, basophils and eosinophils<sup>17</sup>. They also regulate multiple proinflammatory genes encoding cytokines, chemokines and inflammatory enzymes associated with repression of AP1 and NF-Kappa B transcription<sup>18</sup>. Glucocorticoids also inhibit antigen presentation<sup>19,20</sup> and major histocompatibility complex class II expression<sup>21</sup> and antibodies<sup>22</sup>, and favour T helper (T<sub>H</sub>) 1 versus T<sub>H</sub>2 responses<sup>20</sup>. They influence cytotoxic effects via cell death and oxidative stress<sup>23</sup>, have a role in metabolic regulation through glucose utilisation and ATP production<sup>24</sup> and interact with the major neurotransmitters and many secondary neuropeptidergic systems. As such, glucocorticoids modulate emotion and cognition, with key examples being learning ability, performance, emotional perception and mood<sup>25,26</sup>. These interactions also exemplify how glucocorticoid therapy can result in multiple effects including unwanted side effects such as depression<sup>27-30</sup>.

CORT is a homeostatic anticipatory hormone that is secreted by the adrenal glands. Consequently, under basal conditions it is released with a characteristic circadian pattern of secretion with high levels just before waking (start of active cycle), followed by a steady decline down to trough (or nadir) levels during the sleeping or inactive phase; hence anticipating the needs of the body (figure 2A). CORT's daily rhythm is regulated through **indirect projections** from the SCN to the paraventricular nucleus (PVN) of the hypothalamus, which inhibit corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) release during the inactive phase of the cycle<sup>31,32</sup>. Furthermore, to produce appropriate reactivity to physiological, cognitive and emotional stressors, the brain stem and limbic system also modulate HP activity via projections to the PVN<sup>33,34</sup>.

Axonal terminals in the median eminence release CRH and AVP into the **hypophyseal portal system** where they are transported to the pituitary and stimulate pituitary corticotrophs to release adrenocorticotrophic hormone (ACTH). ACTH is released into the systemic circulation and once at the adrenal cortex stimulates the production of CORT. CORT undergoes *de novo* synthesis and release back into the systemic circulation, enabling it to travel to its target tissues and produce its characteristic metabolic, cardiovascular, immunological and cognitive effects<sup>33</sup>. CORT also acts via an autoregulatory negative feedback loop and inhibits HPA activity via effects at the level of the pituitary, hypothalamic PVN and hippocampus<sup>35</sup>.

Further levels of circadian control exist, including splanchnic nerve innervation of the adrenal glands<sup>36</sup>. The adrenal glands receive autonomic (sympathetic) innervation via neuronal projections of the autonomic portion of the PVN<sup>36,37</sup>, which alters adrenal cortical sensitivity to ACTH with a reduction of responsiveness during the circadian nadir. CORT synthesis and adrenal clock gene functioning is also influenced by a light-sensitive mechanism that occurs across the 24 h period, with a shift in **irradiance threshold** according to the time of day. Thus, in animals housed in complete darkness, although high intensity light activated corticosterone at all times of day, lower intensity light had no effect during the subjective day. The adrenal response therefore is dependent on both irradiance and circadian phase<sup>38,39</sup>. The adrenal gland itself also has an autonomous clock that regulates ACTH sensitivity and steroidogenesis, allowing it to fine tune its own homeostatic control<sup>40,41</sup>. Finally, peripheral CLOCK-mediated acetylation of the glucocorticoid receptor can decrease tissue sensitivity to glucocorticoids in a circadian manner<sup>132</sup>.

These circadian fluctuations in activation of glucocorticoid receptors also have important interactions with multiple other crucial homeostatic processes including the transcriptional activity of other genes that respond to glucocorticoids and their corresponding physiological outputs, such as physical activity and body temperature<sup>5,42</sup>. For example, in the rat, glucocorticoid responsive tryptophan hydroxylase

2, a gene implicated in physical activity, temperature and emotional response has a circadian rhythmicity which is abolished by exogenous steroids<sup>43</sup>.

### **[H1] Stress response**

#### **[H2] Acute stress.**

The acute response to stress is a dynamic process that changes over time, starting with **stereotypic behaviours** and then changing to **goal directed behaviours** specific to the stressor, followed by activation of the SAM within seconds and finally recruitment of the HPA axis, with peak levels of cortisol occurring between 15 and 20 minutes after stress onset<sup>44</sup>. These early responses provide increased energy resources and initiate longer term and slower genomic effects that restrain inflammatory and other potentially dangerous responses<sup>45</sup>. The response to the acute stress of cardiac surgery can be seen in Figure 2B<sup>46</sup>. This response is very interesting for several reasons. First, despite the greatly increased levels of cortisol, the pattern of cortisol secretion remains pulsatile. Second, despite initial high levels of ACTH, these rapidly fall to basal levels while the cortisol remains raised. Despite this fall in ACTH levels, small changes in these basal levels of ACTH initiate large pulses of cortisol, indicating a rapidly induced increased sensitivity of the adrenal cortex to ACTH. This effect has now been investigated in reverse translation studies in rats and complemented with mathematical modelling, which enabled the importance of the dynamic adrenal steroidogenic regulatory network to be characterised<sup>47</sup>.

#### **[H2] Chronic stress**

In response to chronic stress, a dynamic change of the ratio of AVP to CRH in the hypothalamic PVN occurs<sup>48</sup> as well as an associated decreased sensitivity to glucocorticoid feedback<sup>49</sup>. Obstructive sleep apnoea is an excellent model of chronic stress (figure 2C). In sleep apnoea, there is a marked increase in the amount of cortisol released during each secretory pulse – which normalises after continuous positive airways pressure (CPAP) treatment<sup>50</sup>. In critical illness, the situation is somewhat different

with the increased levels of cortisol produced by long-term stress being present for the first few days secondary to increased adrenal sensitivity to ACTH and increased cortisol synthesis<sup>46</sup>. During prolonged critical illness, there is a further change in HPA regulation with reduced cortisol metabolism becoming an increasingly important factor in maintaining raised levels of plasma cortisol<sup>51,52</sup>.

## **[H2] Glucocorticoid signalling**

Glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) are the cognate intracellular nuclear receptors for CORTs<sup>53</sup>. The affinity of CORT for MR is approximately five-fold to ten-fold higher than that for GR<sup>54</sup>. Binding of CORT to its receptors leads to either transactivation or repression of genomic transcription as well as more rapid nongenomic effects<sup>55,56</sup>. Non genomic signalling is mediated via classic<sup>57</sup> or membrane bound variants of the receptors<sup>58,59</sup>. The membrane bound variants have lower glucocorticoid affinity than their classic nuclear counterparts<sup>60</sup>. Nuclear MRs are generally constantly occupied during the day and only become unoccupied at the very low levels of CORT found at night in humans or during the day in rodents. As CORT levels rise to a critical threshold (as seen during the circadian peak or following an acute or chronic stress, nuclear GR and membrane associated MR and GR occupation occurs<sup>53</sup>. It is important to note that the one exception to this effect is the hypothalamic SCN, which does not appear to be regulated by circulating CORT<sup>61</sup>. As such, the SCN is the one place where endogenous CORT cannot shift clock function. Whether this feature is due to a lack of corticosteroid receptors or altered chromatin structure is unclear.

A further level of regulatory control is the tissue specificity of GR and MR distribution. GRs are present throughout the brain and peripheral tissues whilst MRs have more limited localisation, predominately being found in cardiovascular tissue, liver and kidneys, as well as corticolimbic regions of the brain<sup>53</sup>. Although GR is present throughout the brain, only the hippocampus, basal ganglia, lateral septum and medial amygdala neurones present a high MR:GR ratio<sup>62</sup>. Since these areas do not express 11  $\beta$ -hydroxysteroid dehydrogenase (11  $\beta$ -HSD) type 2 (discussed in detail in a subsequent section), MR is persistently occupied even during the circadian nadir and it is GR and the fast acting non-genomic



response of the lower affinity membrane-bound MR that responds when CORT levels rise in response to a stressor<sup>28</sup>. This response helps to prepare an individual to respond to a stressor through enhancing synaptic plasticity at a cellular level, which leads to a behavioural change in the form of altered decision making, attentional bias and risk assessment<sup>63</sup>. Although other brain areas can still respond to changes in CORT this will only be through their genomic and nongenomic GR<sup>64</sup>. Consequently, this region specificity in receptor distribution will modify corticosteroid signalling including the activation and termination of stress responses, and are likely to be important for promoting long-lasting adaptive protective mechanisms such as strategic planning, memory storage and consolidation<sup>65</sup>.

Other layers of regulatory control arise through availability of CORT. Firstly, CORT is highly lipophilic, which means that under basal conditions 90% of CORT is tightly bound to the high-affinity, limited capacity protein carrier corticosteroid-binding globulin (CBG)<sup>66,67</sup>. Around 4–5% is loosely bound to albumin and the remaining 5% of CORT is 'free' and biologically active<sup>68</sup>. CBG levels are controlled by a variety of different factors such as genetic variability, liver and thyroid function, nutritional status, inflammation and stress. Additionally, CBG is saturated at fairly low CORT concentrations, including under basal (non-stressed) levels found at the circadian peak. Similarly to CORT, CBG exhibits homeostatic diurnal variation<sup>69</sup>, the resultant effect being a higher proportion of free (and therefore biologically active) CORT during the circadian peak, which accentuates the diurnal profile of free CORT and the response to stress. CBG also acts as a carrier to specific target sites (such as sites of inflammation) and even acts as a protein thermocouple releasing CORT at sites of increased temperature<sup>70</sup>. CBG concentrations are therefore of great importance for the bioavailability of CORT and its ability to act at a tissue level<sup>71</sup>.

The metabolic clearance of CORT occurs through two mechanisms, enzymic degradation in the liver<sup>71,72</sup> and intracellular metabolism by the two iso-enzymes of 11 $\beta$ -HSD, which catalyse the interconversion of active CORT and the inert forms, cortisone (human) and 11-dehydrocorticosterone (rodents). 11 $\beta$ -HSD type 1 is predominately a reductase that catalyses the regeneration of active

glucocorticoids, which amplifies the cellular actions of CORT<sup>72</sup>. It is widely expressed in liver, adipose tissue, muscle, pancreatic islets, adult brain, inflammatory cells and gonads<sup>73</sup>. 11 $\beta$ -HSD type 1 therefore increases tissue levels of CORT and is thought to be a major factor in regulating tissue sensitivity to CORT, especially under conditions that can increase 11 $\beta$ -HSD type 1 activity such as visceral obesity<sup>71</sup>. In addition, 11 $\beta$ -HSD type 1 is thought to mediate many cognitive and metabolic effects of CORT excess. For instance, 11 $\beta$ -HSD type 1 deficiency in rodents protects against age and stress related cognitive decline whilst transgenic over-expression exacerbates cognitive impairment<sup>74</sup>. The other iso-form, 11 $\beta$ -HSD type 2, is a high-affinity dehydrogenase that inactivates glucocorticoids and is particularly important in the kidney, where it protects mineralocorticoid receptors from activation by the much higher concentration of cortisol<sup>72</sup>. More studies are needed to define how tissue ratios of cortisol and cortisone may change during stress and whether this might provide yet another mechanism to guard our homeostatic state.

## [H2] Ultradian rhythm

Underlying the **circadian rhythm** there is a complex and dynamic **ultradian rhythm** comprised of discrete pulses of secretion of both ACTH and cortisol (figure 2A). This pulsatile pattern is produced at an approximate frequency of 60–90 min with increased pulse amplitude and frequency at the circadian peak<sup>75</sup>. Patterns of secretion are influenced by a multitude of factors including genetics, sex hormones, epigenetic influences, environmental stressors and age, which leads to individual diversity<sup>76,77</sup>. Unlike circadian rhythms, the origin of the ultradian rhythm is not from central SCN regulation; rodents with SCN lesions show an isolated loss of circadian rhythmicity, but the underlying pulsatility of CORT remains<sup>78</sup>. Nor does the ultradian rhythm arise as one would expect from a central hypothalamic ‘pulse generator’, as pulses of CRH do not induce downstream pulses of ACTH and CORT<sup>79</sup>. Mathematical modelling techniques were consequently utilised, which predicted that the ultradian rhythm has a sub-hypothalamic origin<sup>61</sup>. Here, a dynamic feedforward–feedback communication exists between ACTH on the adrenal cortex and endogenous glucocorticoids on the

pituitary corticotrophs, together driving ultradian rhythmicity with a natural delay between ACTH activating adrenal MC2 receptors, glucocorticoid biosynthesis and cortisol release (figure 3). This prediction was subsequently confirmed in our rat model<sup>61</sup>.

The adrenal gland itself preferentially responds to oscillatory ACTH signals; in rodents whose endogenous ACTH is suppressed with exogenous glucocorticoids, pulsatile ACTH infusions result in pulsatile corticosterone secretion whereas constant ACTH infusions of the same total dose result in no response<sup>80</sup>. Additionally, the adrenal gland has its own internal feedforward–feedback system that amplifies the response to rapid changes of ACTH, sensitising the adrenal glands to oscillating levels of ACTH and hence alterations in CORT<sup>81</sup>.

## **[H2] Impact of hormone oscillation**

Both circadian and ultradian oscillations in hormone levels matter. Between 5% and 20% of cells show clear circadian oscillations in mRNA levels<sup>12</sup> and circadian fluctuations are found at almost every stage of gene expression<sup>82</sup> and have been associated with important biological effects. In transgenic mice, if circadian peak levels of CORT are administered during a motor learning task, these mice show increased memory of motor skills<sup>83</sup>. This effect arises secondary to a rapid GR-mediated non-genomic mechanism via LIM kinase signalling that increases numbers of new neuronal spines. Low trough levels of CORT are then needed to retain and stabilise the memory. This retention and stabilisation involves ‘pruning’ of old spines via a MR-mediated pathway that alters gene expression, which implies that circadian fluctuations are required for plasticity in learning and memory<sup>83</sup>. Indeed, clinically this phenomenon is nothing new as in patients taking glucocorticoid-based therapeutics and in Cushing syndrome, both situations with chronically elevated glucocorticoid levels and/or no oscillations, there are marked impairments in learning and memory<sup>25</sup>.

What we are beginning to understand is that circadian rhythmicity is only the tip of the iceberg and functionally important dynamic changes are seen on a shorter time frame than simply circadian alterations. In the HPA axis, ultradian hormone presentation is essential for physiological gene

functioning<sup>84</sup>. Both *in vitro* and *in vivo* very different patterns of gene activation are seen depending on the pattern of hormone presentation<sup>85</sup>. Ultradian oscillations in glucocorticoid levels result in a phenomenon known as gene pulsing (figure 4A). This phenomenon is specific for the endogenous glucocorticoid hormones as synthetic glucocorticoids have long binding characteristics at the GR and cannot cycle on and off<sup>85</sup>. As CORT levels rise, as is seen in endogenous glucocorticoid pulsatility, GR binding and activation occurs followed by nuclear translocation, dimerization and interaction with deoxyribonucleic acid (DNA) glucocorticoid response elements (GRE), which initiates transcription. Next, rapid cycling of GR and transcription factors on and off chromatin occurs. Once CORT levels decline, as seen in pulse termination, GR dissociates from DNA and is released back into the nucleoplasm ready to rapidly respond to subsequent CORT pulses. If the same dose of CORT is used but presented as a constant as opposed to pulsatile pattern of presentation, genomic reactivity changes lead to altered transcriptional activity<sup>85,86</sup> as well as differential gene responses (figure 4B)<sup>85</sup>. In addition to the classic GRE-dependent interactions, GR can also interact with a variety of other transcription factors and influence transcription via protein–protein interactions<sup>87,88</sup> and chromatin remodelling<sup>87,89</sup>. These pattern dependent interactions have serious implications for therapeutics based on continuous exposure to glucocorticoids, and is likely to contribute to the adverse effects of these drugs<sup>16</sup>.

Downstream, constant and pulsatile patterns of CORT result in differential neuronal and behavioural responses. For instance, noise stress induces neuronal activation of c-fos in the amygdala, hippocampus and hypothalamic PVN. Interestingly, pulse phase is crucial for this response. If the noise is administered during the rising phase, the c-fos response in the amygdala is increased (Figure 4C). This effect was paralleled by differential behavioural responses to the stressor. Importantly, the effect is reproducible with the pulse phase dependency pattern being recorded in aggressive and novelty behaviour responses to glucocorticoids, as well as in the neuroendocrine mechanism of rapid HPA negative-feedback<sup>90,91</sup>. Translational human studies have found similar results (figure 4D). Healthy male volunteers on a block and replace regimen of endogenous glucocorticoid blockade with

metyrapone and replacement therapy with either circadian or ultradian subcutaneous hydrocortisone or standard oral hydrocortisone therapy (three times a day) at the same total daily dosages exhibit altered cognitive and behavioural effects that are dependent on the cortisol pattern. Specifically, a lack of physiological pulsatility was associated with poorer quality of sleep, poorer working memory performance and altered accuracy in recognition and attentional bias toward or away from emotional faces<sup>92</sup>.

## **[H1] Stress and disease**

Stress is any stimulus that disrupts or threatens homeostatic balance. The stress response is a mechanism that can restore homeostatic processes and promote self-preservation through a complex interaction between the HPA axis, central and peripheral autonomic nervous systems and immune systems<sup>15</sup>. The HPA axis response to stress is by rapidly releasing CORT. As CORT is highly lipophilic it is not pre-stored in vesicles and is synthesised *de novo* in response to ACTH. During long-term stress a dissociation between ACTH and CORT secretion develops<sup>93,94</sup>, predominantly via increased adrenal sensitivity to ACTH<sup>47</sup> or non-ACTH mediated mechanisms<sup>94</sup>. This effect is seen in patients undergoing cardiac surgery, who show a marked shift in adrenal responsiveness (figure 1)<sup>46</sup>, reverse translation animal studies suggest this shift arises secondary to changes in the regulation of both stimulatory and inhibitory intra-adrenal signalling pathways<sup>46</sup>. In models of inflammatory bowel disease and viral challenges, IL-6 and ACTH independent immune–adrenal pathways have also been linked to this dissociation<sup>95,96</sup>.

The rapid rise in CORT seen under conditions of stress increases availability of supplemental energy stores by promotion of gluconeogenesis and inhibition of insulin production combined with vasoconstriction, which aids delivery of blood to muscles and the brain<sup>97</sup>. Centrally, CORT promotes decision making and alertness and stimulates cognitive functioning. CORT also has a pro-inflammatory effect, resulting in an increased response to infection<sup>98</sup>. Chronic exposure to stress results in reversal of the beneficial effects with long-term cortisol exposure becoming maladaptive, which leads to a

broad range of symptoms and disease states, including the metabolic syndrome, obesity, cancer, mental health disorders, cardiovascular disease and increased susceptibility to infections<sup>10,11,53,99,100</sup>.

With regards to immune functioning, components of both the adaptive and innate immune system undergo circadian variations in levels and functionality to optimise response and recovery to a pathogen<sup>101</sup>. Autonomous circadian clocks are also found on different subsets of immune cells, including macrophages and lymphocytes<sup>102,103</sup>. CORT also has a multitude of effects on the immune system<sup>20</sup>. Chronic increases in CORT, either as a result of the effect of the steroid or the development of glucocorticoid resistance<sup>100</sup>, increases susceptibility to infection<sup>20</sup>. Chronic stress can impair GR function as a result of long-term exposure to the resultant release of pro-inflammatory cytokines. Indeed, cytokines (along with their signalling pathways including but not limited to mitogen-activated protein (MAP) kinases, nuclear factor-kappaB (NF-κB) and cyclooxygenase) inhibit GR function<sup>98</sup>. Potential mechanisms underlying this inhibition include disruption of GR translocation, GR–DNA binding, GR inflammatory mediator interactions and alterations in GR phosphorylation status<sup>104-106</sup>. Glucocorticoid resistance in turn interferes with HPA downregulation of the very same pro-inflammatory cytokines and creates a vicious cycle. Thus, in the case of the common cold, an exaggerated response to the symptoms and signs of the upper respiratory tract illness is generated by the resultant pro-inflammatory response<sup>107</sup>.

Long-term exposure to CORT also increases blood levels of glucose<sup>108</sup> and stimulates adipocyte precursor maturation, which promotes adiposity<sup>109</sup>. Adipose tissue is now seen as an active endocrine gland rather than a passive energy store as it produces a wide variety of hormones and regulatory factors<sup>71</sup>. The levels of these factors in turn reflect the metabolic status of the adipocytes and interact with homeostatic connections between adipose tissue and the brain as well as other organs, which together control energy intake and expenditure<sup>71,110</sup>. Obesity is also linked to chronic low-grade inflammation<sup>107</sup> via TNF and IL-6. TNF inhibits intracellular insulin signalling pathways and IL-6 promotes atherosclerosis and metabolism of glucose and lipids<sup>110,111</sup> all of which interact with the HPA

axis<sup>20</sup>. Studies of an increasingly common stress associated problem — sleep deprivation — confirm elevated nadir CORT levels, which then acts as another risk factor for insulin resistance<sup>112</sup>. Behaviourally, chronic sleep deprivation is associated with increased appetite and energy expenditure<sup>113</sup>. Chronic CORT exposure also stimulates mesolimbic reward pathways within the brain, leading to increased intake of palatable food such as sucrose solutions<sup>110</sup>. Additionally, increased feeding might be stimulated by leptin and ghrelin levels<sup>114,115</sup>. During chronic stress it is thought that these two hormones can no longer accurately signal caloric need<sup>116</sup>. The effect in this situation is a misperception of insufficient available energy stores<sup>115</sup>. This effect is in combination with increased pro-inflammatory cytokine levels<sup>28</sup>, which makes it unsurprising that chronic stress and chronodisruption are linked with the development of metabolic complications.

Hypercortisolaemia is also linked with affective disorders<sup>10,27</sup>. Mathematical modelling techniques have shown that individuals with major depressive disorder have disordered cortisol secretion with increased mass of cortisol in each pulse and increased pulse frequency<sup>117</sup>. Patients with major depressive disorder have long been known to exhibit glucocorticoid resistance in the form of resistance of endogenous cortisol to suppression in the dexamethasone and dexamethasone–CRH suppression tests<sup>118</sup>, the results of which can predict clinical outcome<sup>119</sup>. Chronic exposure to stress results in a degree of limbic system atrophy, a key region involved in regulation of emotion<sup>120</sup>. This effect is also closely interlinked with immune function; stress induced proinflammatory cytokines, including IL-1 $\beta$ , IL-6 and TNF, are implicated in depressive behaviour in rodent models of depression and in depressed patients<sup>100,104,121,122</sup>. The mechanisms underlying this are complex. It is possible that inflammation and glucocorticoid signalling may act on the same processes to cause cumulative damage or that the dual pro and anti-inflammatory effects of glucocorticoids disrupt the brain's immune system in favour of inflammatory effects<sup>123</sup>. In patients with depression, decreased T cell availability might arise secondary to increased apoptosis sensitivity combined with impaired glucocorticoid responsiveness and reduced brain trafficking capacity in response to the appropriate neuroendocrine or immune stimuli<sup>100,121</sup>. Stress also enhances NF- $\kappa$ B activation<sup>105,106,124</sup>, indeed stress-

induced anhedonia has been found to be directly linked to increased NF-κB activity<sup>104</sup>. Interestingly, antidepressants can enhance GR functioning<sup>125</sup> and inhibit cytokine pathways<sup>126</sup>.

These effects of glucocorticoids are usually accepted as simply being due to the high levels found in the blood. However, this theory might be an oversimplification. In primary hypocortisolemia (or Addison disease), patients are treated with what we believe to be optimal replacement of hydrocortisone three times a day to mimic the circadian rhythm<sup>127,128</sup>. Despite this effort to give physiological replacement these patients have increased mortality as a result of cardiovascular disease, infectious disease and cancer and increased morbidity from fatigue and difficulties in concentrating<sup>129-131</sup>. This therapy fails to provide the anticipatory rise of cortisol prior to waking and thus gives a shifted circadian profile while also resulting in acute nonphysiological levels of cortisol in the middle of the day<sup>132</sup>. Patients have altered immune responses in the form of altered circadian patterns of circulating monocytes and natural killer cells<sup>133</sup>, impaired natural killer cell function<sup>134</sup> and hypomethylated CD4+ T cells<sup>135</sup>. In an attempt to rectify these adverse outcomes, modified and dual release preparations<sup>133,136,137</sup>, as well as continuous<sup>138</sup> and pulsatile pump (The Pulses study, ISRCTN67193733) preparations are being trialled. Initial results are encouraging, with closer mirroring of circadian profiling resulting in alterations in clock gene function<sup>139</sup> and improvements in immune functioning<sup>133</sup> and quality of life<sup>133,136,140,141</sup>. These issues are not only limited to endogenous CORT, but also synthetic glucocorticoids<sup>16</sup>. These are some of the most widely prescribed medications<sup>142,143</sup> but despite their clinical efficacy over three quarters of patients experience adverse effects even at what would be considered a fairly low dose<sup>144,145</sup>. Chronic glucocorticoid treatment can also induce glucocorticoid resistance in susceptible patients with glucocorticoid responsive conditions such as asthma or rheumatoid arthritis<sup>146</sup>.

## **[H1] Conclusions**

Homeostatic processes are dynamic and interactive. As society changes with development of a 24 h world and its use of social media and changes in interpersonal communication, many of our



evolutionarily adaptive processes are in danger of becoming maladaptive. If we are to understand the growing epidemic of stress-related human disease, we need to go back to first principles and understand not only the mechanisms underlying the regulation of our patterns of physiological activity and hormone secretion — particularly over the important and notoriously difficult nadir period during sleep — but also why they are important for the maintenance of health. If we are going to aspire to an objective of optimal personalised medicine, we need to devise methods not only to measure dynamic basal patterns of hormonal metabolic and immune functioning over the whole day, but also novel therapeutic interventions to counteract the causes of environmental and/or stress related illness.

#### Glossary terms

Zeitgebers - Cues that entrain or synchronise the bodies 24-hour cycle

Circadian clock – Biochemical oscillator with phases synchronised with solar time

Circadian rhythm – Any biological process that displays an oscillation of approximately 24 hours

Ultradian rhythm – A biological rhythm that occurs with a frequency of <24 hours

Indirect projections – Neural pathways involving at least one relay

Hypophyseal portal system – The microcirculation allowing transport of hypothalamic hormones to the pituitary gland

Irradiance threshold – The threshold power of (solar) electromagnetic radiation need to exert an effect

Stereotypic behaviours – Repetitive body movements that serve no biological function

Goal directed behaviours – Behaviour engaged for a specific functional purpose

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#### Author contributions

The authors contributed equally to all aspects of the article.

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**Figure 1 | Co-ordination of central and peripheral clocks by glucocorticoids.** The SCN central clock receives light dark signals which in turn influences HPA and SAM activity leading to circadian CORT production. CORT activates glucocorticoid receptors in peripheral tissues which synchronises peripheral clocks and downstream metabolic, cardiovascular, neuronal and immune pathways. Other zeitgebers such as food, temperature and social cues can also entrain or influence the entrainment of clocks and can alter the output of these downstream pathways.

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**Figure 2 | Human cortisol ultradian rhythmicity under basal and stressful conditions.** Cortisol is well known to be secreted in a circadian rhythm characterised by a nadir or quiescent phase during the inactive period (overnight whilst sleeping in humans). An anticipatory CORT rise occurs before waking followed by the circadian peak of secretion at the start of the active period. Underlying this rhythm, however, is a complex and dynamic ultradian rhythm consisting of pulses approximately every 3 hours. (A) A schematic representation of a circadian and ultradian cortisol rhythm from a healthy male volunteer. (B) The effect of acute stress of cardiac surgery (shaded area shows when coronary artery bypass grafting occurred– taken from Gibbison et al *Critical Care* 2015). Cortisol levels remain elevated throughout the sampling period. This occurs via an initial surge in ACTH, followed post operatively by altered adrenal sensitivity to ACTH. **[Au: Are parts a and b the wrong way around in the figure? That is, the original figure's part c (a here) shows cardiac surgery and**

part d (b here) shows the healthy volunteer? The grey area would then indicate the cardiac surgery – YES grey area is surgery in the original pptx slide C is the cardiac surgery (b) and D is the healthy volunteer (a)?] (C) The effect of chronic stress on HPA axis activity in the form of obstructive sleep apnoea, taken from Henley et al JCEM 2009. Here, disordered cortisol pulsatility is seen in the form of an increase in the number of and amount of cortisol secreted per pulse at baseline (before CPAP therapy), especially over the quiescent period when endogenous cortisol levels naturally fall. The profiles improve after 3 months of continuous positive airways pressure therapy. [Au: As per your figure sketch, I have asked our Art Editor to just include the top 2 panels from the original figure 1b – correct? yes]

Figure 3 | Schematic representation of the HPA axis showing natural inbuilt adrenal delays. After release from the pituitary gland ACTH binds to adrenal Melanocortin 2 receptors. Here natural inbuilt delays occur; MC2R binding leads to increased uptake of cholesterol, the precursor for all steroid hormones. Cholesterol then undergoes a series of biosynthetic steps before being transported into mitochondria, undergoing hydroxylation and the subsequent release of cortisol into the circulation. [Au: Please include a full figure legend, giving more detail on what is depicted in the legend.] [Au: I am unsure that is meant in the delay section of the figure – please could you clarify what you would like us to redraw here? E.g, by labelling the different parts. – new figure supplied]

**Figure 4 | Importance of gene pulsing.** (A) In adrenalectomized rats, hourly pulses of corticosterone result in a similar pattern of association–dissociation kinetics in GR–DNA binding and downstream cyclic recruitment and pulsatile release of the clock gene *Per1* nascent RNA. This effect is not seen with synthetic glucocorticoid treatment (taken from Stavreva et al NCB 2009) B) Ultradian and constant corticosterone treatment have different effects on transcription of *Tsc22d3*, a GR-regulated gene. Here, pulsatile presentation shows a pulsatile pattern of gene fold induction, whilst constant infusion induces constant RNA release (taken from Stavreva et al NCB 2009). C, control; P, induction; W, withdrawal. (C) Adrenalectomised rats received either pulsatile corticosterone (50 ng/ml or 100ng/ml peaks) or constant corticosterone (12-h constant infusion clamped at 50 ng/ml). They then underwent a 10-minute noise stress. C-fos mRNA expression was analysed in different brain regions. A differential response was found in the pituitary, amygdala, hippocampus and PVN. The effect shown was dependent on both phase and amplitude of the glucocorticoid pulse, which suggests that a differential response is seen in stress circuitry depending on the pattern of exposure (taken from Sarabdjitsingh et al Endocrinology 2010). [Au: As per your sketch, I have asked our Art Editor to just

**include part c from the original figure, OK? - YES]** (D) Healthy male volunteers underwent a chemical adrenalectomy with metyrapone. In a cross over trial design they then received hydrocortisone replacement therapy (total daily dose 20mg) as either standard oral (three times a day), constant circadian subcutaneous infusion or constant pulsatile subcutaneous infusion for 5 days. On day 5, participants underwent functional MRI whilst performing a facial recognition task (FERT). Blood Oxygen level Dependent (BOLD) imaging showed a differential response in attentional bias and recognition of emotional faces depending on whether a pulsatile or a non-pulsatile pattern of glucocorticoid presentation was used. Communication networks within the insula, striatum and amygdala also varied depending on whether the presentation was pulsatile or non-pulsatile. (taken from Kalafatakis et al PNAS 2018)