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Salivary cortisol as a non-invasive *window on the brain.*

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Abstract

The validation of accurate and meaningful assessment of cortisol in saliva samples has proved revolutionary in stress research. Its many advantages have expanded the scope of investigation from traditional laboratory and clinical settings to include multidisciplinary and community-based research. These developments have given rise to a wealth insight into the links between stress and health. Here we highlight the potential of salivary cortisol as both a product and mediator of brain function, instrumental in disturbing brain health. However, the subtleties of salivary cortisol as a measure can be underestimated, leading to misinterpretation of findings. These issues are explored, with a particular emphasis on necessary methodological rigour. Notwithstanding great promise, there is undeniably more to learn so we conclude by making recommendations for future research including use of salivary cortisol in the development of integrative predictive models of stress-related risk factors and resilience across the life course.

Why is cortisol interesting?

It is increasingly apparent that stress exerts powerful effects on brain health via a complex and highly coordinated stress-response system (Joëls & Baram, 2009; McEwan, 2012;

McEwen, Eiland, Hunter & Miller, 2012). A better understanding of the causative pathways, individual differences in vulnerability and effective individual and population-based counter-measures to stress is important to diminish its harmful effects. Progress in the investigation of these vital issues requires accurate measurement of relevant variables: salivary cortisol is just such a measure. Changing levels of the hormone cortisol in peripheral body fluids are the product of complex brain processes but if measured accurately and interpreted appropriately can provide valuable insight: a non-invasive indirect *window on the brain.* It can be measured not only under laboratory conditions but also in people going about their everyday lives, providing ecological validity. From hour to hour circulating cortisol concentrations can change in response to everyday thoughts and emotions: negative events (e.g. stress) initiate a spike whereas experiences that are more pleasurable cause a reduction. These variations in cortisol secretion can be accurately captured by measurement in saliva. The cortisol response to stress reflects the function of complex brain networks including the amygdala, frontal cortex and hippocampus. At the same time, levels of cortisol exert powerful effects on the brain's structure and function with chronically high levels linked to neurotoxic effects affecting mood, cognition and the stress response itself. In other words, the hormone cortisol is both a product and mediator of brain function, instrumental in disturbing brain health. This bi-directional role underpins the central importance of cortisol in the analysis of stress and brain health. Much research has been dedicated to analysing the correlates and consequences of the size of the salivary cortisol response to stress and diurnal cortisol patterns in the hope of illuminating biopsychosocial causal pathways, providing a non-invasive biomarker of current brain function and indicator of vulnerability to future brain health. These timely volumes will highlight the role of stress in affecting brain health, interpreting the latest thinking on measurement, mechanisms and potential counter-measures, making them relevant for a wide range of researchers and practitioners seeking insight about future directions and implications for public health.

What is cortisol?

Cortisol is an important and pervasive steroid hormone performing a wide range of 'housekeeping' duties to ensure healthy functioning. As most bodily cells have cortisol receptors it affects multiple and diverse systems, ranging from regulation of the metabolic, immune, cardiovascular and cognitive systems (McEwan, 2000). All of these functions make cortisol a crucial hormone to protect overall health and well-being. It is the product of a neuroendocrine cascade, meaning it is coordinated from the brain via a signalling system known as the hypothalamic-pituitary-adrenal axis (HPA axis). The HPA axis is a key conduit by which the brain can exert control over physiological activity, which it does in normal everyday activity and also in response to stress. The neural control centre for the axis is in the hypothalamus, a region of the brain located below the thalamus within the evolutionary old limbic system, our emotional brain. The paraventricular nucleus (PVN) lies deep within the hypothalamus and is the 'trigger point' receiving neuronal input from various modalities including the cognitive and emotional brain (i.e. sensitivity to stressors) as well as the hypothalamic suprachiasmatic nucleus, transmitting environmental information denoting dark-light transitions that informs the circadian pattern of secretion. In response to activation the parvocellular cells of the PVN secrete the neuropeptide, corticotropin releasing factor (CRF) which in turn stimulates the release of adrenocorticotrophic hormone (ACTH) from corticotrophs in the anterior pituitary, an endocrine gland that sits just below the hypothalamus. Adrenocorticotrophic hormone (ACTH) once released into the general circulation stimulates steroidogenic activity and cortisol release from the *zona fasciculate* of the adrenal cortex. Cortisol (corticosterone in rodents) in common with all of the adrenal hormones is derived from the steroid precursor pregnenolone (itself derived from cholesterol).

In summary, the HPA axis is a signalling cascade from the brain to the adrenal cortex, resulting in cortisol secretion into the general circulation. As cortisol is lipid-soluble once in the blood stream it is able to pass freely through cellular plasma membranes, giving it

access to all cells, including the brain. Within the brain cortisol's effects are widespread, dependent upon two types of receptor which differ in their distribution and properties: the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Cortisol exerts effects on the brain through both genomic (directly binding to DNA) and non-genomic mechanisms, affecting neurotransmitters, neurotrophic factors, sex hormones and other stress mediators to shape present and future responses to stress (Gray, Kogan, Marrocco, & McEwen, 2017). The MRs are localised in the limbic system and prefrontal cortex, binding cortisol with high affinity, whereas GRs, which are more widely distributed throughout the brain, and bind cortisol with approximately one tenth of the affinity (de Kloet, Meijer, de Nicola, de Rijk, and Joëls, 2018). MR activation is related to the onset of the stress response whereas GR are associated with facilitation of recovery. The distinct difference in receptor type, distribution and sensitivity allows cortisol to regulate brain function in different ways depending on ambient concentrations: it is the balance between receptor occupancy that determines outcomes (de Kloet, Joëls, & Holsboer, 2005). As reviewed in these volumes the stress response and its feedback is complex, having many and diverse effects on brain structure and function. Salivary cortisol concentrations represent the net effect of these brain processes, underpinning its validity in the study of stress and brain health.

Stress and its measurement

Within engineering stress refers to physical strains that lead to structural distortion. This terminology is adopted for living things: biological and psychological disturbance caused by events is known as stress, whereas the stress-inducing events are referred to as stressors. Obviously, there are vital differences between the engineering concept of stress and human stress. Not least of these is the predictability of the relationship between stressor and stress. The engineer can have confidence in a foreseeable relationship between stressor and stress; one steel girder will behave much like the next. This is not the case for human stress

where the stressful experience is not directly proportionate to the degree of stressor. For example, a relatively minor stressor might have a large effect on biology and behaviour; alternatively, potent external stressors might not necessarily induce proportional stressful reactions, thus human stress is subjective. Psychologists have been studying the cause of these individual differences for years and emphasize the mediating role of appraisal, coping, and emotion: depending on goals, beliefs, and coping strategies, a stressor may be appraised differently and elicit different stress reactions between and within individuals. A large part of the salivary cortisol literature deals with this issue including the role of pre-natal, early life development and environmental influences that can affect vulnerability and resilience to stressors, all of which are discussed in this volume. In addition, our reactions to stress can change across the lifespan and there are things we, as individuals and communities, can do to counteract the negative effects of stressors on our systems.

Stress can be defined and objectively measured in terms of its biological response. This response is composed of two distinct but interacting physiological systems: the sympathetic nervous system (SNS) with noradrenaline as the key mediator, and the HPA axis with cortisol as the main downstream hormone. Whilst the SNS is not uniquely activated by threat i.e. stress (it is also activated by excitement and other forms of positive arousal) activation of the HPA axis is fine-tuned to react to threat and is therefore a ‘cleaner’ indicator of stress than study of the SNS alone. Together the stress-response systems allow for adjustment to a wide range of bodily and environmental demands and in this way are adaptive and beneficial for outcomes. Stress-related problems for the brain and body arise when stress-reactions are persistent and sustained – known as ‘chronic stress’. Accordingly, in the short-term stress response systems are adaptive but in the long-term, under sustained activation they become maladaptive, leading to ill-health.

It is interesting to consider that the stress-response to psychosocial threat is equivalent to that to physical threat. So, the stress-response machinery that facilitates the appropriate

energy for the flight and fight response (necessary if we are confronted by an assailant, for example) is totally out of proportion for dealing with common psychosocial stress (e.g. difficult relationships, inadequate housing, pressure and lack of control at work). Standardised exposure to short-term psychosocial stress in the laboratory increases salivary cortisol levels (Dickerson & Kemeny, 2004). It is very interesting to examine the most reliable stimuli for generating substantial stress response are threat to self-esteem, lack of control and novelty. Sadly, today exposure to these stimuli can be frequent everyday events that can result in harmful repeated stress responding i.e. chronic stress, and ultimately affect brain function and health outcomes. Of course, the experience of stress can affect broader function than these two response systems. For example, behaviour is usually affected, often leading to maladaptive stress coping mechanisms such as smoking and drinking alcohol. Sleep and appetite are frequently disrupted whilst mood, memory and thinking styles can be modulated. These additional stress responses can also be measured, but for the purposes of studying stress and brain health the primary focus of investigation is the role of the HPA axis as the primary instrument of change.

Salivary cortisol measurement in human studies

For many years cortisol was typically only measured in humans from single measure blood samples or 24-hour urine collections for use in clinical diagnostics. Clemens Kirschbaum, working in the celebrated biological psychology research group of Dirk Hellhammer in Germany first validated accurate and meaningful assessment of cortisol in saliva samples (see Kirschbaum and Hellhamer 1989). This step proved revolutionary due to its many advantages over blood and urine, taking investigation of cortisol secretion away from the clinical setting in to a realm of multidisciplinary research. It opened up opportunities to investigate the dynamically changing state of biologically relevant HPA axis activity from repeated sampling of easily accessed saliva. In the bloodstream, most cortisol (about 90%) is stored as a reservoir bound to specific binding proteins transcortin and albumin. Once

bound in this way it is transported around the body but is not biologically active. Only unbound cortisol is 'free' as it is available for receptor binding making it biologically active. Assessment of cortisol in blood samples measures **total** cortisol with no distinction between bound and free. Without accurate knowledge of how much is bound to the proteins, it is impossible to estimate the biologically active component without undertaking the complicated process of osmosis, which separates the two components in a test tube. In contrast, assessment of salivary cortisol measures **only** the biologically active 'free' component of cortisol as the bound component is too large a molecule to pass through the saliva glands. Another substantial advantage of using saliva as the medium in which to measure cortisol is that passage through the saliva glands is passive, not an active transport system, as for salivary IgA, for example. This means that the concentration of cortisol in saliva is not dependent on salivary volume, in other words secretion does not have to be calculated per unit time, which would make it methodologically much more challenging. Any volume of saliva collected over any length of time (so long as the volume is adequate for the assay) can generate accurate measures of biologically active cortisol, representative of that circulating through the entire body at any one time. These advantages together with sampling convenience and ease of repeated sampling, even outside of research settings provides huge opportunities for neuroendocrine research.

Stress reactivity studies

A commonly used approach is to examine the scale of cortisol responding following exposure to a standardised psychosocial stressor, such as the Trier Social Stress Test (Kischbaum, Pirke, and Hellhammer (1993). Responses interact with a wide range of demographic and psychosocial variables (Kudielka, Hellhammer and Wust, 2009; Zänkert, Bellingrath, Wüst, Kudielka, 2018) making it problematic to associate different cortisol responses with specific patterns of brain activation (Dedovic, Duchesne, Andrews, Engert, Pruessner 2009). It is interesting however that hyper- and hypo-responding of the HPA axis are associated with distinct negative future health outcomes (Turner et al, 2019) suggesting

they are associated with distinct patterns of brain activation. Exaggerated reactivity predicts greater risk of coronary artery calcification, hypertension and more rapid telomere attrition. In contrast, blunted HPA axis reactivity predicts more depressive and PTSD symptomatology, greater musculoskeletal pain and lower bone mineral content (Turner et al, 2019). These findings, implicating both exaggerated and blunted cortisol responding to stress, are problematic as there are no guidelines for the optimal 'or goldilocks' stress response (i.e. not to large or too small). This complication reinforces the need to be vigilant to include appropriately matched healthy control groups from which to compare populations of interest.

Blunted stress-induced salivary cortisol secretion is an interesting index of dysregulated HPA axis function as, amongst other things; it has been associated with neuroticism and impulsivity, which are characteristic of various behavioural disorders. Although it remains a developing story, it appears to be an indicator of Reward Deficiency Syndrome, reflecting fronto-limbic dysregulation (Carroll et al, 2017). Optimal responses to stress occur within a normal range (the 'goldilocks' zone) and deviation in either direction signals poor systems integration. Adequate cortisol stress reactivity is purportedly protective for brain health via mediation of reward system activity (Oei, Both, van Heemst, & van der Grond, J., 2014), which is consistent with blunted cortisol reactivity to stress in clinical depression (Burke, Davis, Otte, & Mohr, 2005). Of course, studies of stress reactivity with peripheral markers, such as salivary cortisol, cannot pinpoint the precise site of brain dysregulation, but they can provide useful insight by revealing a deviation from the 'norm' worthy of further investigation.

This area of research is also complicated by the issue of non-responding. Within any experimental stress reactivity study a notable percentage of participants (e.g. 35%, Smyth et al, 2019) will not mount a salivary cortisol response, defined as either a 1.5 nmol/l or 15.5% percentage baseline-to-peak increase (Miller, Plessow, Kirschbaum, & Stalder, 2013). A lower criteria (i.e. 1 nmol/l) might apply depending on the assay used for cortisol

determination (see Miler et al., 2013). The issue of non-responding has been relatively ignored but in our data of healthy females we have not observed any association between demographic or psychosocial variables and non-responding (Smyth et al, 2019). Incidence of non-responding in males, who typically exhibit larger responses (Herbison et al., 2016), has not been reported. Non-responding is assumed to be random, attributed to the timing of the stressor coinciding with the refractory phase of the individual underlying ultradian rhythm. Rodent studies have demonstrated that if stress exposure coincides with the falling phase of the ultradian burst both the behavioural and neuroendocrine response to stress is markedly attenuated (Lightman & Conway-Campbell, 2010; Sarabdjitsingh, et al, 2010), although this has not been examined in humans. As non-responding is suggested to be random, not a form of blunted HPA axis reactivity, it is recommended to present data analyses with and without their inclusion (Miller, Plessow, Kirschbaum, & Stalder, 2013). In addition, the prevalence and characteristics of non-responders should be reported to inform data on their characteristics and enable transparent monitoring of data handling.

Basal pattern of salivary cortisol secretion

The basal patterns of cortisol secretion reveals insight about brain processes involved in circadian regulation, which are vital for brain health (Karatsoreos et al, 2011; Oster et al, 2016). However, it has proved surprisingly tricky to tease out the role of stress-related cortisol secretion in brain health precisely because of the changing levels of cortisol over the day. Cortisol secretion is subject not only to perceptions of stress (i.e. stress-responsive) but also to the brain's internal clock, which synchronises a characteristic 24-hour (circadian) pattern. This input from the suprachiasmatic nucleus provides the basal platform of cortisol secretion on which stress reactivity is superimposed, and sustained stress over a period of time ultimately dysregulates this cycle. Healthy salivary cortisol concentrations change about 20-fold per day/night cycle, providing an important chemical signal to downstream processes about appropriate activity for the time of day, and it is this that makes cortisol essential for

daily functioning. The cortisol circadian cycle acts as the conductor of a physiological orchestra ensuring disparate body systems remain aligned for maximum efficiency and healthy flourishing of the whole being i.e. integrated healthy functioning. There are beneficially high levels of cortisol after awakening, known as the cortisol awakening response (CAR: Preussner et, al, 1997) and a decline from morning (about 30 min post-awakening) until bedtime, known as the diurnal decline and lowest levels during the early phases of sleep. Unfortunately, the significance of this circadian cycle was overlooked in many early studies, compromising experimental rigor. It is now apparent however that measurement of this methodologically problematic circadian cortisol cycle in saliva is itself a potent *window on the brain*, as its disruption by chronic stress is an early indicator of dysregulated brain function and the major route by which stress affects brain health (Adam et al, 2017; Gianaros et al, 2017). Aberrant patterns of cortisol secretion may not always translate into concurrent observable symptoms but the reason they are interesting is that, if sustained for any length of time, they provide evidence of causality and a useful early indicator of negative future health outcomes. As such, study of this hormone in saliva samples provides a useful pre-clinical indicator of the links between mind and body in currently healthy and clinical populations (Smyth, Hucklebridge, Thorn, Evans, and Clow, 2013).

The ultradian pattern of cortisol secretion and the cortisol awakening response

Cortisol secretion is dynamic, subject to negative feedback that induces an oscillating secretory pattern, with short-lived secretory bursts followed by intra-pulse intervals of approximately one hour. This means that HPA axis function is pulsatile with the resultant pattern of cortisol secretion known as the ultradian rhythm. The amplitude (to a less extent the rate) of pulses varies throughout the day, with a peak after awakening and a trough at sleep onset, providing the basis of the overarching circadian pattern. Hourly bursts of cortisol secretion are evident in peripheral body fluids of individuals when using frequent (or

continuous) blood sampling. They are not apparent in less frequent sampling protocols and when data from multiple individuals is summed to reveal overall trends. The importance of pulsatility in HPA axis function is attracting increasing attention with the pulses (rather than overall concentrations) shown to influence GR-mediated behavioural and neuroendocrine responses to stress, circadian clock genes, glutamatergic transmission and synaptic plasticity in the hippocampus (Fitzsimons et al. 2016; Flynn, Conway-Campbell, & Lightman, 2018). It is not surprising therefore, that disruption of the pulsatile ultradian rhythm is implicated in a range of neuropathology (Fitzsimons et al. 2016). Evidently it is important to be aware of this interesting and important area; however, analysis of hormone pulsatility is not easy in saliva samples as the individuals within group data will have pulses at slightly different times resulting in the mean concentration removing evidence of the underlying ultradian rhythm. More recently, interest has focused upon the cortisol awakening response (CAR) as the first pulsatile event of the day (Evans, Smyth, Thorn, Hucklebridge, & Clow, 2019).

The CAR is usually measured from saliva samples and is necessarily measured relative to the moment of awakening, a time described as the ‘tipping point’ of the day i.e. the moment when the brain switches from night-time sleep to daytime consciousness (Clow, Hucklebridge, Stalder, Evans, Thorn, 2010). The process of awakening is thought to synchronise the start of the ultradian rhythm so that the CAR, even measured in saliva in multiple participants, is apparent as the coordinated first pulse of the day. Later in the day (more remote from the awakening tipping point), even small inter-individual differences in ultradian timing smooth out all evidence of pulsitility. The underlying ultradian rhythm might also be why some people do not show a CAR i.e. awakening during a refractory phase (as for non-responding in stress reactivity studies, discussed above). We have found evidence of this in nearly 20% of days despite accurate sampling, Smyth et al., 2013, however, no study has examined if this non-CAR is a ‘trait’ characteristic. Studies measuring CAR on repeated days (up to a week) are required to examine this.

The CAR is the only time in the circadian cycle when cortisol levels rise peaking at around 30-45 minutes post awakening. This means that study of the CAR, from saliva sampling, may provide even more insight into brain function than the rest of the diurnal cycle as it provides insight into not just an index of circadian but also ultradian function. Consistent with this it has recently been proposed that CAR salience i.e. the shape, including the rate of cortisol decline following the peak is a key measure, indicative of the salience of the underlying ultradian pulse (Evans, Smyth, Thorn, Hucklebridge, & Clow, 2019). This is a unique feature of the CAR and may account for its sensitivity as a biomarker in stress research (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010). It is proposed that the CAR (as an ultradian marker) has a particular role in synchronisation of peripheral (or 'slave') clocks throughout the body (Clow, Hucklebridge, Stalder, Evans, Thorn, 2010). It is clear that, stress-related flat diurnal declines are associated a wide range of physical and mental health (Adam et al, 2017). Similarly, stress-associated flattening of the CAR has been shown to be associated with reduced hippocampal integrity and volume and impaired brain function (Law and Clow, in press; Clow et al, 2014; Shi et al, 2018). It is proposed that this is due to compromised synchronisation between the central clock in the hypothalamic suprachiasmatic nucleus (via the CAR) and slave clocks in the peripheral tissue, including brain (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010). Such lack of synchronisation is known to impact brain health (Menet & Rosbash, 2011). Certainly, this intriguing possibility needs further exploration as if true provides a powerful tool in neuroendocrine research and a potential target for intervention for a wide range of brain conditions.

Alternative strategies to probe brain function using salivary cortisol

Of course, measures of salivary cortisol are limited; they do not provide a literal picture of brain function. Instead, if judiciously applied and interpreted, these measures provide valuable *indirect* clues of current brain function and an early warning signal of possible

forthcoming neurotoxic effects. However, some strategies can probe more deeply. For example, the dexamethasone suppression test is a tried and tested way of revealing brain cortisol receptor sensitivity. Non-suppression of salivary cortisol concentrations indicates that the administered glucocorticoid was unable to shut down the HPA axis generation of new cortisol, and this is a finding prevalent in clinical depression (Carroll, 1982; Arana, Baldessarini, & Ornsteen, 1985). Similarly, metyrapone is used to probe sensitivity of the HPA axis by temporarily inhibiting the hydroxylation of 11-deoxycortisol into cortisol, effectively blocking cortisol biosynthesis. Such an approach has been used, alongside salivary cortisol monitoring to explore the role of the CAR in memory retrieval (Rimmele, Meier, Lange, Born, 2010).

To date the primary focus of salivary cortisol research has been to inform causative pathways linking stress and brain health. Opportunities now exist for applying this knowledge to evaluate stress-reduction strategies, at the individual and community level. As reviewed in these volumes much promising intervention work has been undertaken but unfortunately evaluation strategies can be methodologically flawed (Smyth, Rossi & Wood, in press). Introduction of simple measures of basal levels of salivary cortisol (if properly collected, see below) can provide valuable objective insight into change, even in clinical conditions (e.g. Smyth et al, 2019). Such evidence lends weight to the effectiveness, or not, of interventions – not just as a remote biomarker but as a significant instrument of changing brain health. The important message here is that measures of salivary cortisol are responsive to changing environmental and individual circumstances and that these changes reflect changing brain health in terms of HPA axis status and potential neurotoxic effects.

Methodological issues when measuring basal salivary cortisol secretion

The multiple advantages of using saliva as a sampling medium from which to measure cortisol secretion have made it overwhelmingly popular in multidisciplinary research.

However, the advantages also bring methodological challenges. The primary issue is around the marked circadian nature of basal cortisol secretion. Self-collection of samples has opened up research into areas not previously possible and been able to capture daily life, with ecological validity. The simplicity has enabled sampling from virtually any participant group from tiny infants to diverse and disabling clinical conditions. Such studies typically seek to capture a picture of the daily (diurnal) pattern of cortisol secretion asking for repeated sampling at different times across the day. The moment of morning awakening i.e. the transition from sleep to consciousness, is the starting point in each 24-hour cycle. It begins with a synchronised surge in cortisol secretion reflecting the first ultradian pulse of the day (i.e. the CAR). This pattern means that all assessment of cortisol secretion should be synchronised to the individual time of awakening. It is not adequate to use clock time, as there are large individual differences in awakening time (Edwards. Clow, Evans, & Hucklebridge, 2001). This means that accurate timing of sampling when using self-collection study designs is of critical importance for measurement of morning levels.

Accurate assessment of the CAR has proved to be the most methodologically problematic area in salivary cortisol research. It is demanding of study participants to collect samples immediately on awakening and at 15 min intervals up to 30-45 min post-awakening (without eating or having a cup of tea/coffee). To successfully capture the moment of awakening and self-collect saliva samples at the correct time intervals and in the correct tubes is difficult, especially when suffering from considerable sleep inertia. Sampling inaccuracy leads to erroneous measurement of the CAR (Smyth, Clow, Hucklebridge, Thorn, Evans, 2013). However, errors can be minimised if electronic measurement of the sampling process are used (Smyth, Thorn, Hucklebridge, Clow, & Evans, 2016). The CAR is also subject to considerable day-to day variability, in response to state variation (Law Hucklebridge, Thorn, Evans & Clow, 2013) requiring measurement on repeated days in order to assess an average i.e. trait-like CAR (Hellhammer et al, 2007). These issues have led to much debate in the area leading to expert consensus guidelines that are **essential** reading for all potential

researchers of this particular aspect of cortisol section (Staldler et al, 2016). The effort involved in CAR measurement is considerable. If it is not possible to follow the published guidelines it is best to focus upon measurement of the diurnal decline as a measure of basal cortisol secretion (Adam et al, 2017). This approach should avoid inclusion of the CAR period and sample between 3-6 times over the post-awakening day, up until the evening.

Conclusions

This chapter highlights the potential of using measures of salivary cortisol as a bi-directional stress-related indicator of brain health. As reviewed in these two volumes cortisol is both the product of a stress response system and capable of having profound effects on brain structure and function. Study of the developmental and psychosocial predictors of stress responding can provide some insight into the causal origins of such dysregulated brain function. Similarly, examination of the underlying basal circadian pattern of cortisol secretion, especially the CAR, sheds a light on how well this vital neuroendocrine system is working. Efficient circadian/ultradian systems are essential for health, regulating disparate physiological processes, including brain function. Flattening of the circadian pattern of cortisol secretion is an indicator of disrupted circadian function and can be detected even before signs of aberrant brain function appear, providing the opportunity for monitoring and early intervention. Additionally, the sensitivity of salivary cortisol secretion to stress-alleviating interventions makes it a valuable marker of effectiveness.

Clearly, use of salivary cortisol in the investigation of brain health has great potential. However, there is undeniably a lot more to learn in order to interpret different cortisol profiles in relation to specific indices of brain health. Crucially, the area suffers from a dearth of work using salivary cortisol in large-scale population based prospective studies to test its value as a mediator between stress and brain health. A recent meta-analysis reported just 10% of published studies used a longitudinal methodology (Adam et al, 2017). Use of salivary cortisol measures alongside a range of brain health/behavioral outcomes, multimodal

neuroimaging and network neuroscience in both males and females would also do much to inform and facilitate future utility. A vital aim must be to build integrative predictive models (Stringer & Tommerdahl, 2015) to identify stress-related risk factors across the life course, as well as protective factors. Inclusion of salivary cortisol in such models would provide an accessible and valuable adjunct to the study of brain health and resilience.

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