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Quantifying the Autonomic Response to Stressors—One Way to Expand the Definition of "Stress" in Animals

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Synopsis Quantifying how whole organisms respond to challenges in the external and internal environment ("stressors") is difficult. To date, physiological ecologists have mostly used measures of glucocorticoids (GCs) to assess the impact of stressors on animals. This is of course too simplistic as Hans Seyle himself characterized the response of organisms to "noxious stimuli" using multiple physiological responses. Possible solutions include increasing the number of biomarkers to more accurately characterize the "stress state" of animal or just measuring different biomarkers to more accurately characterize the degree of acute or chronic stressors an animal is experiencing. We focus on the latter and discuss how heart rate (HR) and heart rate variability (HRV) may be better predictors of the degree of activation of the sympathetic–adrenal–medullary system and complement or even replace measures of GCs as indicators of animal health, welfare, fitness, or their level of exposure to stressors. The miniaturization of biological sensor technology ("bio-sensors" or "bio-loggers") presents an opportunity to reassess measures of stress state and develop new approaches. We describe some modern approaches to gathering these HR and HRV data in free-living animals with the aim that heart dynamics will be more integrated with measures of GCs as bio-markers of stress state and predictors of fitness in free-living animals.

Introduction

There is a growing appreciation that measures of "stress" are problematic. In vertebrate physiological ecology and ecological physiology, researchers have almost exclusively relied on glucocorticoids (GCs) for assessing how stressors impact animals (Romero et al. 2015; MacDougall-Shackleton et al. 2019). For example, in a review of how to measure "stress" in wildlife using measures of GCs, Sheriff et al. (2011) stated that "Measuring GC levels does not equate to measuring 'stress', but they are a critical component of the stress response and, when taken together with other indices of stress (e.g., measures of immune function, metabolism, nitrogen balance), they offer considerable insight into how animals perceive and adapt to their environment." Measuring GCs is nonetheless useful because they seem to play a major role in facilitating organismal resilience through environmental challenges (Sapolsky et al. 2000; Vitousek et al. 2018). However, measuring only GCs is problematic because of its simplicity as the stress response is multifaceted and composed of autonomic, neuroendocrine, and behavioral responses. In fact, GCs are not necessarily symptomatic of stress, nor do they reliably predict individual animal fitness in nature (Breuner 2008; Bonier et al. 2009; Crespi et al. 2013; Dantzer et al. 2016).

Other than altering our terminology and verbiage so that we are more careful to not equate "an increase in GCs" with "an increase in stress levels," where do we go from here? One solution is to make our measures of "stress" more multifaceted than they have been in the past. Interestingly, this is similar to what Seyle (1936, 1943, 1956) described when he characterized the stress response as the "general adaptation syndrome" or "general alarm reaction." Seyle measured not just one feature of the stress response but the "syndrome" that was composed of multiple physiological responses and endpoints indicative of exposure to noxious stimuli, which can be synonymous with a disease state. The suggestion to expand how we quantify the stress response has been called for recently. For example, Breuner et al. (2013) highlighted the need for other

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metrics to quantify exposure to chronic stress such as glucose or free-fatty acid levels or the production of heat shock proteins. Dickens and Romero (2013) emphasized that there is not one single physiological variable that one could use to characterize individuals as being "under chronic stress." Recent studies (e.g., Romero et al. 2009) have also endeavored to quantify the multitude of effects stressors have on behavior, the hypothalamic–pituitary–adrenal axis, and the sympathetic–adrenal–medullary system.

Those of us that study free-living animals have been historically constrained by the field environment to the extent that concurrent toolsets are difficult to implement. Thus, the reliance on GCs as an accessible and proximate measure of stress has become prominent, though flawed. The miniaturization of biological sensor technology ("bio-sensors" or "bio-loggers") presents an opportunity to reassess measures of stress and develop new approaches—potentially animal-borne—that can be united with the vast work on measures of GCs in free-living animals. One such route of investigation focuses on the brain–heart axis as they are bi-directionally connected to the sympathetic–adrenal–medullary system. Here, the electrical activity of the central nervous system (CNS) modulates the catecholaminergic tone onto the heart, causing changes in heart rate (HR) and heart rate variability (HRV, Fig. 1). This link between the brain and the rest of the body is potentially significant with respect to stress. We aim to describe the physiological mechanisms involved in the sympathetic stress response, their significance in animal physiological ecology and ecological physiology, and present modern approaches to gathering these data in free-living animals. In doing so, we hope to present a balanced comparison, and perhaps, integration of heart dynamics with GCs as biomarkers of stress and predictors of fitness in free-living animals.

The autonomic stress response

The common caricature of the immediate response to adversity or environmental challenges ("fight or flight") is accompanied by a cascade of physiological changes. In mammals, the limbic system is highly conserved and serves as a neural substrate for fears and emotions (Jänig 1985), controlling sympathetic outflow systems to eventually activate the cardiac muscle and adrenal glands (Jansen et al. 1995; Porges 1995; Chapleau and Abboud 2001). This is how and why GCs remain a valid surrogate measure for stress, and although the effect of GCs on the body are relatively slow, they play an important

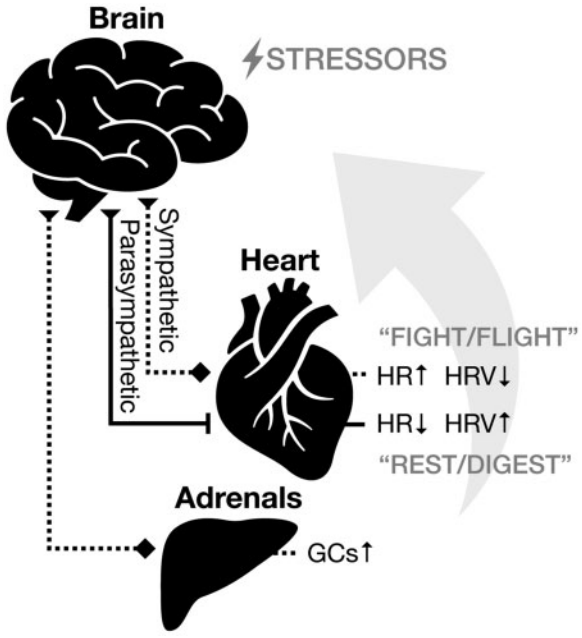

Fig. 1 Challenges in the external and internal environment ("stressors") modulate cardiac rhythms through descending autonomic pathways. Sympathetic branch activity is indicative of exposure to stressors and causes an increase in HR and a decrease in HRV, while parasympathetic branch activity decreases HR and increases HRV. GCs can also increase in response to exposure to acute or chronic stress.

role in responding and adapting by regulating glucose production and temporarily suppressing the immune system (Padgett and Glaser 2003). The autonomic stress response is not only mounted during distress, but also disease states that challenge homeostasis beyond a typical range for the species.

Immediate autonomic effects are mediated by catecholamines, namely, epinephrine and norepinephrine, which agonize ß-receptors on the heart enhancing contractility while increasing HR (Lacombe and Jones 1990; Cyr and Romero 2009). ß-blockers compete with endogenous catecholamines to reduce HR (Amer 1977), which is associated with positive clinical outcomes (Arnold et al. 2008) suggesting that HR, rather than the presence of catecholamines, is deleterious. Meanwhile, the parasympathetic pathway (i.e., "rest and digest") is inhibited, which is why the stress response is said to be "sympathetic-dominant." Immediate changes to HR also occur during breathing, as HR accelerates during inhalation due to the inhibition of vagal outflow, which is restored via the release of acetylcholine following exhalation (Eckberg and Eckberg 1982).

Prolonged activation of the sympathetic system can be due to emotional and neural dysregulation, environmental uncertainty, consistent threats,

lingering noxious stimuli, or irregular breathing, and should ultimately be detrimental and maladaptive. For example, one of the best cardiac risk factors in humans remains an elevated HR (>90 beats per minute) due to sympathetic over-activation (Curtis and O'Keefe 2002; Zhang et al. 2016) and similar, negative implications of an elevated HR have been found in other animals (Umana et al. 2003).

How stress affects HR

HR has historical significance as one of the best measures to assess the health and behavioral status across taxa (Levine 1997). HR is often used to characterize the autonomic response to stress as it can reflect the balance between the sympathetic and parasympathetic systems that elevate and depress HR, respectively. The autonomic influence over HR has been directly tested by co-administering a ß-blocker (propranolol) and anticholinergic (atropine), therefore exposing the spontaneous HR generated intrinsically by the sinoatrial node (Jose and Collison 1970). Somewhat surprisingly, the heart beats faster when the autonomic inputs are blocked in this fashion, suggesting that the parasympathetic branch, which depresses HR through the vagal nerve, is normally dominate (Levy and Zieske 1969; Uijtdehaage and Thayer 2000). In contrast to sympathetic activation, these findings have led researchers to alternatively focus on vagal "tone" as a health indicator (Levy and Schwartz 1994).

In humans, free-living birds, and mammals, HR responds to acute stressors (e.g., an immediate, novel, or unpredictable stimulus) in a very similar way, although direct comparisons are difficult and often necessitate captivity. In wild birds, anthropogenic disturbances such as exposure to humans can elevate HR (Viblanc et al. 2012a, 2015) as can exposure to agonistic interactions between neighbors (Viblanc et al. 2012b). Black bears (*Ursus americanus*) have a significant increase in HR associated with the perceived threat of road crossings (Ditmer et al. 2018). Similar elevations in HR are found in captive birds and mammals where restraint or noxious stimuli are presented (Nephew et al. 2003; Ellen et al. 2014). The startle response is also associated with an immediate elevation in HR following the disturbance (Young and Leaton 1994; Johnson and Mayers 2001; Nephew and Romero 2003; Laferton et al. 2018).

How chronic stress impacts HR is less clear. For example, 4 weeks of exposure to a chronic stress paradigm causes sustained elevations in baseline HR in laboratory rats (Grippo et al. 2003). In female prairie voles (*Microtus ochrogaster*), social isolation is a type of chronic stressor and voles experiencing 4 weeks of social isolation exhibited substantial increases in resting HR (Grippo et al. 2007; Grippo and Johnson 2009). Stress imposed by capture elevates HR for the first 30 h but continues to affect the HR-associated startle response for >10 days suggesting blunted reactivity (Dickens and Romero 2009; Fischer and Romero 2016). Interestingly, in a separate study that used a chronic stress paradigm that exposed subjects to four different types of stressors for 30 min every 1.5–2 h, the daytime increase in HR during 15–16 days of exposure to this chronic stress paradigm was met with a nighttime decrease during that same period (Cyr et al. 2009). However, this counterbalancing effect to a chronic stressor (wounding) was abolished when the birds were molting (Durant et al. 2016), which in itself may be a stressful life history stage in birds as it is often associated with elevated GCs.

Although HR may be a useful measure to characterize the autonomic response to stress and some studies in captive animals (described above) illustrate that exposure to chronic stressors may elevate resting HR, there are certain methodological caveats associated with measuring HR that are illustrated by studies in humans. For example, heart rhythm is still one of the first vital signs examined by a physician, but as we expect with other species, it is often not interpreted without greater context (e.g., did the patient drink a cup of coffee in the waiting room?). Even then, the prognostic quality of HR itself is tenuous in clinical medicine. In humans, a typical standard deviation of HR can be up to 10 beats per minute, which is not itself significantly different from some disease conditions (Albanese et al. 2016). Additionally, an elevated HR in humans can also reflect healthy behavior (e.g., physical exercise) and HR associations with chronic health conditions like depression are mixed, sometimes showing higher resting HR (Krittayaphong et al. 1997) and sometimes lower (Hu et al. 2016). Co-existing psychophysical conditions (Thayer et al. 2010; Licht et al. 2011) and socioemotional conditions (Watkins et al. 1998) are not met with the same physiological responses as physical stressors in humans, and there may be unique coping mechanisms in primates (Cameron and Schoenfeld 2018). The problem with using HR as a window into the stress state of an animal is, therefore, that it is challenging from a taxonomic, environmental, circadian, and life history perspective, as they are all factors that affect HR and HR-associated characteristics (Viblanc et al. 2015). Therefore, a cardiac measure that provides

more resolution into the underlying physiology is required and may be found in HRV.

HRV as a better indicator of stress state

In 1965, it was found that the inter-beat interval (IBI) of the heart was an earlier predictor of fetal distress than HR itself (Hon and Lee 1963). Overlapping with the rise of accessible computing power, statistical measures of HRV were soon pioneered (Akselrod et al. 1981). By the late 1980s, HRV gained clinical relevance as a detector of autonomic neuropathy in diabetic patients (Ewing et al. 1985) and as a strong predictor of mortality following an acute myocardial infarction (Wolf et al. 1978; Kleiger et al. 1987; Malik et al. 1989; Bigger et al. 1992). Since then, there has been a relatively lengthy body of research showing that HRV is an accurate measurement of the activity of the autonomic stress response (Thayer et al. 2012) and can signify a state of heightened vigilance (Thayer and Lane 2000). A reduction in HRV (i.e., a more regular heartbeat) is a result of vagal withdrawal and sympathetic activation (Schiweck et al. 2019) characteristic of exposure to stressors (Koolhaas et al. 1999; Perini and Veicsteinas 2003; Stauss 2003; von Borell et al. 2007; Cyr and Romero 2009), which can result in or indicate unfavorable health outcomes. The regularity between heartbeats under sympathetic dominance is likely advantageous for health and survival, as it guarantees a consistent blood circulation and delivery of nutrients and glucose to peripheral organs. However, maintaining such a mode of operation may be biophysically maladaptive, as it can render the organism impervious to changing circumstances (Thayer and Sternberg 2006). An autonomic blockade not only increases HR but also decreases HRV in humans (Camm et al. 1996) and rodents (e.g., Cyr et al. 2008; Lakin et al. 2018) highlighting the important influence of vagal tone on regulating HRV.

Across species, exposure to acute or chronic stressors is associated with a reduction in HRV. For example, transportation or acute restraint (e.g., during grooming) of agricultural animals is associated with a reduction in their HRV (Reefmann et al. 2009; Schmidt et al. 2010). Lameness in cows (characterized as abnormalities of the feet that cause pain when moving and may lead to infection and sepsis) is a type of chronic stressor and cows that exhibit lameness had lower HRV than those that did not exhibit these symptoms (Kovacs et al. 2015). In laboratory rats, 4 weeks of exposure to a chronic stress paradigm resulted in reductions in HRV (Grippo et al. 2003). Female prairie voles exposed to social isolation for 4 weeks exhibited reductions in HRV (Grippo et al. 2007; Grippo and Johnson 2009). It is less clear how stress and HRV are related in non-mammalian species although many findings are consistent (Fischer and Romero 2016; Müller et al. 2017). For example, acute stress (trauma associated with surgery) in snakes almost eliminates HRV for the first 10 days following surgery (Sanches et al. 2019). In contrast, in studies in captive birds exposed to a chronic stress paradigm for 16 or 18 days, HRV was unaffected (Cyr and Romero 2009; Kostelanetz et al. 2009).

In humans, HRV decreases with age (Reardon and Malik 1996; Padgett and Glaser 2003) and HRV decreases with many pathophysiological conditions including heart failure, diabetes, and hypertension (Xhyheri et al. 2012) as well as obesity (Mazurak et al. 2016). It is unclear how mental stress manifests in non-humans, however, patients with depression have a lower HRV (Krittayaphong et al. 1997) which is unrelated to existing cardiovascular disease (Carney and Freedland 2009) and worsening symptoms further decrease HRV (Krittayaphong et al. 1997; Kemp et al. 2010). HRV is negatively correlated with exposure to stressful experiences (Porges 2003; Stauss 2003; von Borell et al. 2007) and self-reported anxiety (Berntson and Cacioppo 2004) or increased work-related stress (Chandola et al. 2008; Thayer et al. 2010). Experimental application of standardized psychological stress tests (e.g., Stroop or speech task paradigm) to humans also decreases their HRV during wakefulness (Delaney and Brodie 2000) and subsequently during sleep (Hall et al. 2004).

Compared to individuals with high resting HRV, those with low resting HRV do not recover as quickly from psychological stressors based on cardiovascular, endocrine, and immune markers (Weber et al. 2010). One meta-analysis showed that poor recovery following laboratory stressors is associated with cardiovascular risk status (e.g., elevated blood pressure, hypertension, clinical cardiac events), although subjects also exhibited heightened reactivity (Chida and Steptoe 2010 and see below). Indeed, biological responses to stressors or threats can be exaggerated, leading to anxiety or aggression (Valiente et al. 2003; Carthy et al. 2010). For example, studies on severely depressed individuals are mixed, albeit consistently atypical, showing both higher reactivity or a blunted response to stressors (Hamilton and Alloy 2016; Schiweck et al. 2019) similar to the inverted U performance-arousal curve of the Yerkes–Dodson Law (Yerkes and Dodson 1908; Cohen 2011). Physical exercise is one way to

increase resting HRV while establishing normal/optimal autonomic reactivity (Kiss et al. 2016). Unlike psychological stressors, transient activation of the autonomic system from exercise is followed by an augmentation of vagal tone that is positively associated with health (Pardo et al. 2000).

Many of these results support the emerging theory that HRV is a window into the reactiveness and integrative capacity of the CNS to deal with challenges and coordinate context-specific responses in the periphery (Thayer et al. 2012). From this view, a low HRV is going to indicate a maladaptive state, which makes it broadly applicable, if not more nuanced in its interpretation. The origin of changes to HRV has been challenged by the fact that the sinoatrial node is also plastic (Stein et al. 2002). Between cohorts of sedentary and trained mice that show marked differences in resting HR/HRV, heart rhythms after atrial denervation become indistinguishable, supporting the former hypothesis that the CNS primarily modulates HRV through the nervous and related systemic pathways that converge on the heart (Lakin et al. 2018). Indeed, HRV is part of a physiological feedback and feedforward network affected by nervous, immune, metabolic, and endocrine systems (Ernst 2017a) and taken together, heart rhythms (and HRV in particular) may act to compress complex physiological processes into a single node that is accessible and resolute, highlighting its practical utility.

Bridging the gap between GCs and HRV

Given that most studies to date have focused on measuring "stress" using only GCs, it is useful to briefly look at the associations between GCs and HRV. Some studies that employ captivity as a chronic stressor show that the period following initial captivity is associated with an increase in GCs and HR and a decline in HRV but as the time from initial captivity increases, GCs and HR decline and HRV increases (Dickens and Romero 2009). Following the transportation of agricultural animals (a type of acute stressor), GCs are elevated and HRV is reduced (Schmidt et al. 2010). However, other studies find no association between HRV and measures of GCs or even the opposite association where both GCs and HRV are elevated. For example, lameness in cows (a type of chronic stressor) was associated with reduced HRV but no change in fecal GC metabolites compared to non-lame cows (Pacifici et al. 2015). In wild birds brought into captivity, HRV and plasma GCs were reduced at the beginning of captivity compared to 6–7 days after captivity was initiated (Fischer et al. 2016, 2018). These latter studies suggest that the lag time from the initiation of the stressor (in these studies it was captivity) affects their impact on HRV or GCs.

When should HRV and GCs be measured? Fischer et al. (2018) give one example where they sampled blood in the morning to quantify GCs and then split HRV recordings into daytime and nighttime, reflecting a typical design for ecological research. However, blood sampling is itself at-first novel and will remain disruptive to normal behavior in animals, potentially influencing subsequent HRV measurements. Studies in humans testing the stress response typically sample before and after intervention but might also have 24-h recordings to split analyses between morning, day, and night (Kim et al. 2018). This approach is nuanced, considering that HRV does not directly correlate with the cortisol waking response (Stalder et al. 2011), suggesting GCs and HRV are not inherently connected and may be more influenced by circadian rhythms at specific time points. The most general recommendation for baseline measurements is that GCs should be sampled with minimal intervention, before a stress response can permeate the sampled tissue and HRV should be taken while the subject is supine, but not moving (typically, first thing in the morning). Interpreting GCs and HRV may also benefit from the context of the three-stage response model originally proposed by Selye (1956). That is, how the alarm, resistance, and exhaustion stages of the stress response communicate different information about the state of an animal. Testing this model will require detailed data on HRV and GCs in the same individual measured at these three different time points.

Tools and methods to measure HR and HRV

As a relatively young field of investigation, the tools and methods used to record HR and HRV are actively undergoing standardization, which may account for conflicting results (Ernst 2017a). An electrocardiogram (ECG, sometimes called an EKG from the German word *Elektro-kardiographie*) is the gold standard method for measuring heart rhythms and requires an amplifier and electrodes to be strategically placed near the heart muscle. Ample resolution on an ECG will provide information about atrial and ventricular depolarization and repolarization with ventricular depolarization being the largest and sharpest deflection in the signal (also called an "R wave"). The IBI (inter-beat interval) is synonymous with the normal-to-normal R-R interval (NN)

and is the basis for calculating time-series measures like mean NN interval (i.e. HR), as well as statistical operations that are used in HRV analyses.

HR and HRV measures can be affected by a subject's head or body position, respiration rate or pace, sex, age, and aerobic fitness level, and interrelate with natural biophysical rhythms (e.g., circadian, metabolic, hormonal) as well as nutritional supplementation or medication (Ernst 2017b). Clinicians and researchers alike should be aware of the relatively extensive list of best practices and caveats when approaching HR and HRV measurements and subsequent analyses that are discussed elsewhere (Camm et al. 1996; Shaffer and Ginsberg 2017). Here, we briefly introduce the potential utility of HRV analyses based on "short-term" 5-min recordings, as this is a well-documented standard, reasonable to achieve in animals from battery-powered devices, and applicable to 24-h recordings that are chunked into smaller time windows.

Common time-series HRV measures include the standard deviation of all NN intervals (SDNN, measured in ms), the root mean square of successive differences of the NN interval (RMSSD), and the percentage of adjacent NN intervals that differ by >50 ms (pNN50, which can be used as pNNx for other species or alternative analyses; Camm et al. 1996). SDNN measurements reflect both sympathetic and parasympathetic activity, but in short-term recordings, the primary source of variation is parasympathetically-mediated respiratory sinus arrhythmia, making this measure extremely sensitive to respiratory status (Shaffer et al. 2014). RMSSD and pNN50 are correlated with each other and closely with parasympathetic activity. Although time-series analyses are conceptually straightforward, they fail to correlate with the same measures over 24 h (Shaffer and Ginsberg 2017), making their interpretation context-dependent. Studies have also found that short-term recordings are prognostically insufficient (Kleiger et al. 2005).

Analyses in the frequency-domain may offer more insight for short-term recordings. This often begins by subjecting the time-series data (i.e., the entire 5 min) to a form of spectral analysis where the power contributions from different frequency bands can be viewed in two dimensions (power × frequency). A generalized approach has been to quantify low-frequency (LF, 0.04–0.15 Hz) and high-frequency (HF, 0.15–0.4 Hz) power which are correlated with sympathetic and parasympathetic tone, respectively (Xhyheri et al. 2012). Therefore, the LF/HF ratio has been suggested as an index of the interaction between sympathetic and vagal activity (Pagani et al. 1986), but this notion has been challenged, primarily because the LF band is multifaceted. For example, during resting conditions, LF power represents baroreflex rhythms, and may only approximate sympathetic tone when subjects are ambulating (Shaffer and Ginsberg 2017).

Indeed, a sliding window on data chunks can maintain a time-series representation of HRV and be applied to time and frequency domain analyses. These are analytically similar approaches yet serve different disciplines, as physiological ecologists will likely have less data with more electrophysiological artifacts (e.g., due to movement) than a clinician in a controlled setting. Luckily, many software packages assist in this process while performing an array of HRV measures on a variety of data formats (Vest et al. 2018).

Toward a future of bio-loggers recording HR and HRV

The use of miniaturized bio-loggers to make physiological and environmental measurements from free-ranging animals has radically transformed scientific capabilities. Technological strides in battery, computation, memory, and sensor technology continue to support a rich suite of bio-logging tools that are not only becoming smaller and longer-lasting, but providing multi-featured, high-resolution data (Williams et al. 2019).

Stressors imposed by natural environments may better approximate the physiological capabilities of an animal to respond and adapt than stressors applied in the laboratory (Williams et al. 2016). However, quantifying the multifaceted stress response using an attachable, or implantable bio-logger is inherently challenging. Some of the first attempts in this vein used collar-based accelerometers on large herbivores to identify behavioral patterns in response to stressors (Kröschel et al. 2017). Accelerometry has also been used to map micro-movements onto specific internal (Wilson et al. 2008) or disease states (Cancela et al. 2014; Downey et al. 2017). Self-contained, automated hemodynamic measurement units have been used to sample blood during a physical challenge to analyze changes in GCs and other hormones (Landry et al. 2014; Takei et al. 2016). The dynamic relationship between HR and respiration has been characterized using bio-loggers in diving birds (Butler and Woakes 1979) and again examined in penguins with devices capable of identifying unique body functions such as defecation (Wilson et al. 2004). HR also accurately predicts energy expenditure (Weimerskirch et al.

2002), metabolic rate (Green 2011), and circadian phase (Sim et al. 2017) and both HR and HRV are becoming widely appreciated surrogates of stress and resilience (see above). Therefore, HR/HRV may be more capable at resolving the underlying physiological responses to stressors that result in less obvious changes in the behavioral response to stress (e.g., from accelerometry alone).

Some bio-loggers perform on-board HR estimation (Bevan et al. 1997; Chaise et al. 2017; Pulopulos et al. 2018) which is more efficient from a power and memory standpoint, but limits HRV analyses. Beat-to-beat signals are useful for time-series analyses, as the periodically measured RMSSD of IBI in free-ranging, pregnant horses correlate with changing seasons, which may be due to environmental, pregnancy, or metabolic pressures (Pohlin et al. 2017). However, to perform proper short-term HRV spectral analyses the entire, raw waveform must be analyzed, and to our knowledge has so-far relied on post hoc, rather than on-board computation in battery-powered bio-loggers (although this is not true for consumer 'wearables' designed for humans). For example, bar-headed geese (*Anser indicus*) have been fitted with bio-loggers that record long-duration, raw ECG patterns during a trans-Himalayan migration (Spivey and Bishop 2014).

It should be recognized that bio-loggers have additional constraints compared to biotelemetry systems, where data are transmitted rather than directly saved (Fu et al. 2011), and wireless charging might be an option (Yoon et al. 2004). Telemetered heart rhythms have been applied to mammals (Arnold et al. 2004; O'Mara et al. 2017), birds (Cyr et al. 2008), fish (Cooke et al. 2004), and reptiles (Butler et al. 2002). Telemetry systems have outpaced bio-loggers in both capability and use across model systems, although there are notable synergies, and indeed mixed capabilities, as in the form of marine "pop-up" tags (Musyl et al. 2011), RFID-enabled devices (Williams et al. 2016), and Bluetooth low energy devices (Berkvens et al. 2018). Bio-loggers that can record neural data with the resolution to identify single action potentials (>20 kHz) have been implemented in behaving animals with the option to perform short-term experiments where animals are free of a recording tether (Massot et al. 2019). Brain rhythms that characterize different sleep states can be recorded with much slower sampling rates (Rattenborg et al. 2008; Aulsebrook et al. 2016), and potentially ex-vivo using electroencephalography (EEG), thereby extending the lifetime and utility of neuro-based bio-loggers. The discovery that great frigatebirds (*Fregata minor*) sleep mid-flight was facilitated by the availability of bio-loggers that constantly recorded EEG (at 200 Hz) over 10 days (Rattenborg et al. 2016). Similar devices have been implemented in pigeons and represent a powerful toolset when paired with other onboard sensors and behavioral synchronization systems (Vyssotski et al. 2006). Indications that sleep states affect physical performance and recovery (Shapiro et al. 1981) and that stress has a bidirectional relationship with sleep (Hall et al. 2004; Martire et al. 2019) makes the neural toolset an exciting new direction for bio-logging technology. HR/HRV may be a key marker for the efficiency and effectiveness of autonomic regulatory processes like sleep and can, therefore, be examined in association with health, survival, and reproduction.

The future of bio-logging to quantify how animals respond and recover from exposure to stressors may rely on smarter and more clever recording techniques to mitigate power and memory constraints (Woakes et al. 1995; Clark et al. 2009; Spivey and Bishop 2014; Cox et al. 2018). For example, accelerometry and time-of-day data could augment ECG recording routines, as some cardiac measurements are only relevant following a period of rest or in the morning (Shaffer and Ginsberg 2017). Brain rhythm data not only pair well with accelerometry data for the ability to distinguish between active, rest, and sleep state but could also coordinate low-power modes on the bio-logger so that the neural recording circuitry is idle when the animal is moving. Although onboard computation is power-intensive, algorithms that perform HRV statistics (Park et al. 2018), detect sleep states (Allocca et al. 2019), extract neuronal spiking rates (Dragas et al. 2013), or discretize any other physiological variables could be valuable depending on the recording strategy. If bio-loggers co-evolve with modern portable electronics, on-board and dedicated artificial intelligence hardware is a near-term strategy to enhance data intelligence and compression of resulting data and potentially prolong battery life.

Researchers aiming to use bio-loggers to collect HR and HRV in free-living animals so as to measure their stress state will also have to develop creative ways to validate their measures of HR and HRV. It is now commonplace to biologically validate assays that aim to measure GCs or their metabolites in non-blood (fecal, hair, feathers, urine, etc.) using some type of physical stressor or pharmaceutical challenge (Touma and Palme 2005). However, we should hold the same standard to those using bio-loggers to quantify the autonomic response to

stressors. This could easily be accomplished by using bio-loggers to measure the effects of captivity and the surgical implantation of the bio-logger (both of which are stressors) on HR and HRV, as has been done in previous studies described above. This may require that a few individual animals are studied intensively or in captivity when the bio-loggers are first deployed.

The largest challenge to monitoring HR and HRV in free-living animals is that it can be more invasive than the collecting of blood samples and other tissues that can be used to measure GCs non-invasively (feces, urine, feathers, hair). However, they may provide much higher quality and informative data than measures of GCs alone and benefit from an accelerating technological ecosystem that spans many organisms (e.g., Aimie-Salleh et al. 2019). Moreover, they can provide high resolution data on a smaller number of individuals, thereby emphasizing the "reduce" of the three "R's" of vertebrate animal research (replace, refine, reduce). Researchers must also consider whether intervening on either end of an experiment to implant and explant a bio-logger is altogether less disruptive to the population and other species in the community than the consistent or frequent presence of humans, which is often required to collect samples to measure GCs.

Conclusions and why should we measure HRV?

HRV appears to occur in all vertebrate taxa (*sensu* Sanches et al. 2019) and there are several similarities to studies that use measures of GCs to measure how an animal perceives its world and the degree of environmental challenges it is facing. For example, GCs (Schoenle et al. 2018) and HRV (Muller et al. 2018) both show repeatable individual differences, suggesting that these traits can exhibit an evolutionary response to natural selection. Although there are challenges associated with measuring HRV compare to GCs, we think there are several clear advantages. First, GCs play a role in the mobilization of glucose to fuel behavioral activities and so elevated GCs may be a biomarker of elevated energetic expenditure in wild animals. However, by measuring HRV, measures of HR are also available. It is quite likely that measures of HR better reflect actual energetic expenditure than measures of GCs given that they can be used to estimate oxygen consumption and therefore energetic expenditure (Groscolas et al. 2010; Ellenberg et al. 2013), although they require careful validation (e.g., Hicks et al. 2017). Second, measures of GCs have been used to investigate how anthropogenic activities impact wildlife (Dantzer et al. 2014) but measures of HR, and perhaps HRV, in wild animals may provide a more in-depth view of how they affect wildlife. For example, wild animals may not exhibit a behavioral response to anthropogenic activities but still exhibit an increase in HR (Ditmer et al. 2015). Measures of HR may also provide insights into the unexpected impacts of humans on animals such as the presence of wildlife photographers provoking a stronger increase in HR in penguins than did the capture and handling (Ellenberg et al. 2013). Finally, as we noted above, HRV may better reflect the stress state of an animal as well as the amount of acute or chronic stressors the animal has been exposed to compare to measures of GCs. This is reflected by the ability of HRV to predict human health (Thayer and Lane 2000; Lane et al. 2009) and health/welfare in other animals (von Borell et al. 2007). For example, there is a strong interest in understanding how social interactions affect the health and fitness of all animals including humans ("sociality-health-fitness" nexus). Interestingly, social interactions may increase oxytocin (Uvnas-Moberg 1998) and experimental administration of oxytocin increases HRV (Romero et al. 2014). Moreover, a social isolation stressor applied to prairie voles reduces their HRV but these effects are abolished if oxytocin is administered during the period of isolation (Grippo and Johnson 2009). These studies suggest one way by which social interactions increase health, well-being, and fitness in animals through its effects on oxytocin and HRV.

Studies of the relationship between HRV and measures of fitness in wild animals are clearly needed here but these previous studies suggest that HRV may more reliably predict past or current exposure to stress and may more reliably predict fitness. The obvious limitation here is that measuring HR and HRV is still difficult and much more invasive to the individual compared with measures of GCs. However, a comprehensive picture of the stress response to environmental challenges, as Seyle advocated, is going to require data on the autonomic stress response in addition to measures of GCs and HRV may be the most reliable biomarker.

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